(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 November 2002 (28.11.2002)

PCT

(10) International Publication Number
WO 02/094289 A1

(51) International Patent Classification[7]: A61K 31/708, 31/7076, 31/7068, A61P 31/14

(21) International Application Number: PCT/EP02/05340

(22) International Filing Date: 15 May 2002 (15.05.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0112617.6 23 May 2001 (23.05.2001) GB

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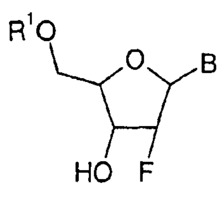
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTIVIRAL NUCLEOSIDE DERIVATIVES



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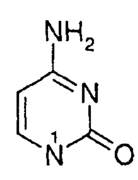

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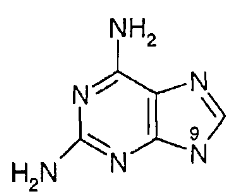

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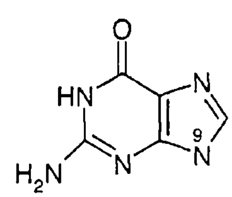

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(57) Abstract: The present invention relates to nucleoside derivatives for use in the treatment or prophylaxis of hepatitis C virus infections. In particular, the present invention relates to known 2'-deoxy-2'-fluoro nucleoside derivatives and their use as inhibitors of hepatitis C virus (HCV) RNA replication and pharmaceutical compositions of such compounds. The compounds of this invention have potential use as therapeutic agents for the treatment of HCV infections. The present invention describes the use of 2'-deoxy-2'-fluoro nucleoside derivatives of formula I wherein R^1 is hydrogen or phosphate and B signifies a 1-pyrimidinyl or 9-purinyl residue of formulae B1, B2, B3 and of pharmaceutically acceptable salts thereof for the treatment of diseases mediated by the hepatitis C virus (HCV) or for the preparation of medicaments for such treatment.

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Antiviral Nucleoside Derivatives

The present invention relates to nucleoside derivatives for use in the treatment or prophylaxis of hepatitis C virus infections. In particular, the present invention relates to known 2'-deoxy-2'-fluoro nucleoside derivatives and their use as inhibitors of hepatitis C virus (HCV) RNA replication and pharmaceutical compositions of such compounds. The compounds of this invention have potential use as therapeutic agents for the treatment of HCV infections.

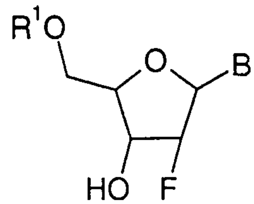
Hepatitis C virus is the leading cause of chronic liver disease throughout the world. Patients infected with HCV are at risk of developing cirrhosis of the liver and subsequent hepatocellular carcinoma and hence HCV is the major indication for liver transplantation. Only two approved therapies are currently available for the treatment of HCV infection (R.G. Gish, Sem.Liver.Dis., 1999, 19, 35). These are interferon-α monotherapy and, more recently, combination therapy of the nucleoside analogue, ribavirin (Virazole), with interferon-α.

Hepatitis C virus belongs to the family of Flaviridae. It is an RNA virus, the RNA genome encoding a large polyprotein which after processing produces the necessary replication machinery to ensure synthesis of progeny RNA. It is believed that most of the non-structural proteins encoded by the HCV RNA genome are involved in RNA replication. Lohmann et al. [V. Lohmann et al., Science, 1999, 285, 110-113] have described the construction of a human hepatoma (Huh7) cell line in which subgenomic HCV RNA molecules have been introduced and shown to replicate with high efficiency. It is believed that the mechanism of RNA replication in these cell lines is identical to the replication of the full length HCV RNA genome in infected hepatocytes. The subgenomic HCV cDNA clones used for the isolation of these cell lines have formed the basis for the development of a cell-based assay for identifying nucleoside analogue inhibitors of HCV replication.

2'-Fluoronucleoside analogues are described in WO 99/43691 as being useful in the treatment of hepatitis B infection, hepatitis C infection, HIV and abnormal cellular

proliferation, including tumours and cancer. 2'-Deoxy-2'-fluoro ribonucleoside derivatives are not described specifically.

The present invention describes the use of 2'-deoxy-2'-fluoro nucleoside derivatives of formula I

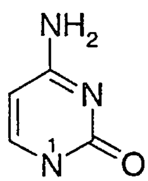


I

wherein

R^1 is hydrogen or phosphate and

B signifies a 1-pyrimidinyl or 9-purinyl residue of formulae B1, B2 or B3



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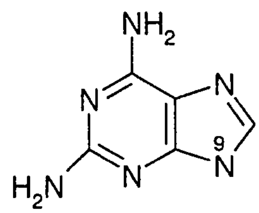

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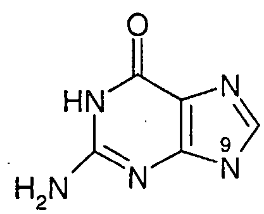


B3

and of pharmaceutically acceptable salts thereof for the treatment of diseases mediated by the hepatitis C virus (HCV) or for the preparation of medicaments for such treatment.

The term "phosphate" as used herein for R^1, denotes a monophosphate, diphosphate or triphosphate group of the formula $-[P(=O)(OH)O]_nH$, wherein n is an integer selected from 1, 2 and 3. Phosphate in R^1 is preferably a monophosphate group. The term "phosphate" further includes stabilized monophosphate prodrugs or other pharmaceutically acceptable leaving groups which, when administered in vivo, are capable of providing a compound wherein R^1 is monophosphate. These "pronucleotides" can improve the properties such as activity, bioavailability or stability of the parent nucleotide.

Examples of substituent groups which can replace one or more of the hydrogens in the monophosphate moiety are described in C. R.Wagner et al Medicinal Research Reviews, 2000, 20(6), 417 or in R. Jones and N. Bischofberger, Antiviral Research 1995, 27, 1. Such pronucleotides include alkyl and aryl phosphodiesters, steroid phosphodiesters, alkyl and aryl phosphotriesters, cyclic alkyl phosphotriesters, cyclosaligenyl (CycloSal) phosphotriesters, S-acyl-2-thioethyl (SATE) derivatives, dithioethyl (DTE) derivatives, pivaloyloxymethyl phosphoesters, para-acyloxybenzyl (PAOB) phosphoesters, glycerolipid

phosphodiesters, glycosyl lipid phosphotriesters, dinucleosidyl phosphodiesters, dinucleoside phosphotriesters, phosphorodiamidatescyclic phosphoramidates, phosphoramidate monoesters and phosphoramidate diesters.

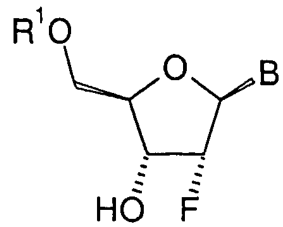
The invention also includes pro-drugs or bioprecursors of the parent nucleoside which are converted *in vivo* to the compound of formula I wherein R^1 is hydrogen or physiologically acceptable salts thereof. Preferred pro-drug derivatives include carboxylic ester derivatives of the 3'- or 5'-hydroxyl group in which the non-carbonyl moiety of the ester group is selected from straight or branched alkyl (e.g. methyl, n-propyl, n-butyl or tert.-butyl), alkoxyalkyl (e.g. methoxymethyl), araalkyl (e.g. benzyl), aryloxyalkyl (e.g. phenoxymethyl), aryl (e.g. phenyl optionally substituted by halogen, C_{1-4} alkyl or C_{1-4} alkoxy or amino); sulphonate esters such as alkylsulphonyl or arylsulphonyl (e.g. methanesulphonyl); amino acid esters (e.g.L-valyl or L-isoleucyl) or pharmaceutically acceptable salts thereof. The preparation is carried out according to known methods in the art, for example methods known from textbooks on organic chemistry e.g. from J. March (1992), "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure", 4th ed. John Wiley & Sons).

In the pictorial representation of the compounds given throughout this application, a thickened tapered line (━) indicates a substituent which is above the plane of the ring and a dotted line ('''''') indicates a substituent which is below the plane of the ring.

Compounds of the present invention exhibit stereoisomerism and therefore include compounds wherein the carbon atoms have the S, R, or R,S-configuration. The compounds of this invention can be any isomer of the compound of formula I or mixtures of these isomers. The compounds and intermediates of the present invention having one or more asymmetric carbon atoms may be obtained as mixtures of stereoisomers which can be resolved, at the appropriate steps in the process of this invention by stereospecific methods known in the art to obtain a given stereoisomer or pure enantiomer having a desired stereoconfiguration. Alternatively, the desired isomers may be directly synthesised by methods known in the art.

In a preferred embodiment of the invention the ribofuranoside is a α-D, β-D, α-L or β-L ribofuranosyl ring, more preferred a β-D or β-L ribofuranosyl ring, and most preferred a β-D ribofuranosyl ring.

The preferable relative configuration of compounds of this invention is that of formula I-a,



I-a

wherein

R^1 and B are as defined above, and of pharmaceutically acceptable salts thereof.

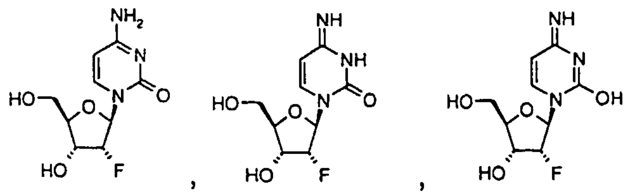
Compounds of formula I exhibit tautomerism (as known from textbooks on organic chemistry e.g. J. March (1992), "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure", 4th ed. John Wiley & Sons) that means that the compounds of this invention can exist as two or more chemical compounds that are capable of facile interconversion. In many cases it merely means the exchange of a hydrogen atom between two other atoms, to either of which it forms a covalent bond. Tautomeric compounds exist in a mobile equilibrium with each other, so that attempts to prepare the separate substances usually result in the formation of a mixture that shows all the chemical and physical properties to be expected on the basis of the structures of the components.

The most common type of tautomerism is that involving carbonyl, or keto, compounds and unsaturated hydroxyl compounds, or enols. The structural change is the shift of a hydrogen atom between atoms of carbon and oxygen, with the rearrangement of bonds.

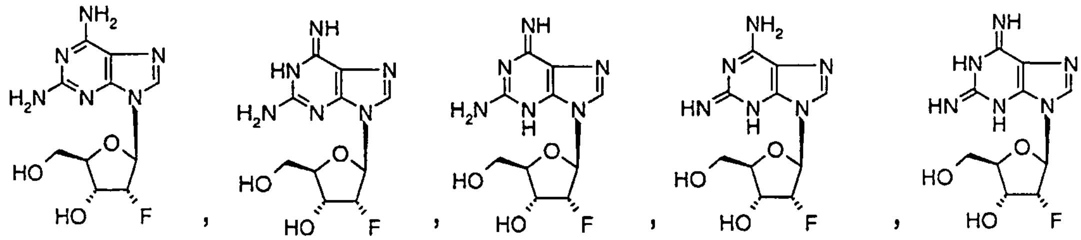
For example, in many aliphatic aldehydes and ketones, such as acetaldehyde, the keto form is the predominant one; in phenols, the enol form is the major component. An intermediate situation is represented for example in ethyl acetoacetate, which at room temperature contains about 92.4% keto and 7.6% enol; at –78°C, the interconversion of the two forms is slow enough for the individual substances to be isolated.

It will be appreciated that within the present invention compounds of formula I exist in various tautomeric forms and that they are encompassed by the present invention. Preferred tautomeric forms are drawn below:

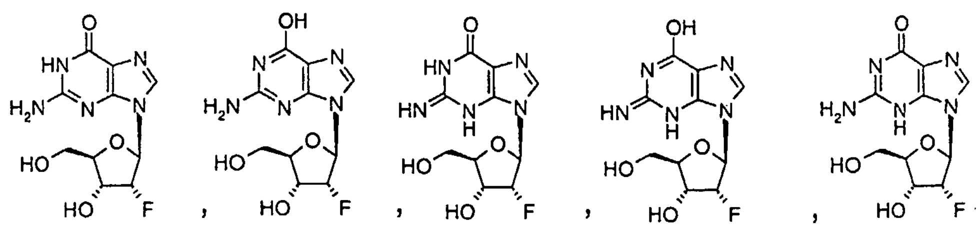
2'-Deoxy-2'-fluorocytidine:



2-amino-2'-deoxy-2'-fluoroadenosine:



2'-deoxy-2'-fluoroguanosine:



The above compounds preferably exist in the form drawn first.

Compounds of formula I which are basic can form pharmaceutically acceptable salts with inorganic acids such as hydrohalic acids (e.g. hydrochloric acid and hydrobromic acid), sulphuric acid, nitric acid and phosphoric acid, and the like, and with organic acids (e.g. with acetic acid, tartaric acid, succinic acid, fumaric acid, maleic acid, malic acid, salicylic acid, citric acid, methanesulphonic acid and p-toluene sulphonic acid, and the like). The formation and isolation of such salts can be carried out according to methods known in the art. Compounds of formula I which are acidic can form pharmaceutically acceptable base salts derived from appropriate bases such as alkali metals (e.g. lithium,

sodium, potassium), alkaline earth metals (e.g. calcium, magnesium), ammonium or NX_4^+ (wherein X is C_{1-4} alkyl, preferably methyl or ethyl, more preferred methyl).

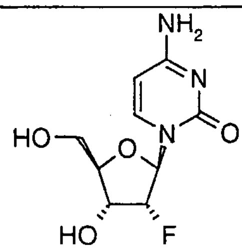
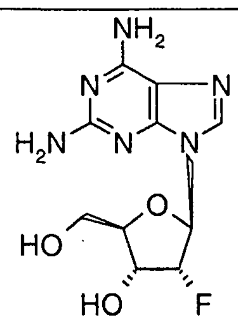
A preferred embodiment of the invention is the use of compounds of formula I or I-a as defined above wherein

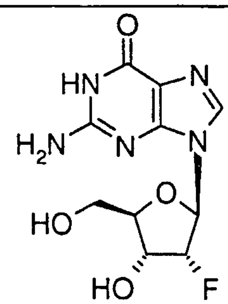
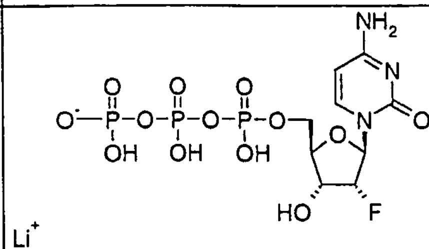
R^1 is as defined above and B signifies 1-pyrimidinyl,

and of pharmaceutically acceptable salts thereof.

More preferred embodiments of compounds of formula I for the use in the treatment of diseases mediated by the hepatitis C virus (HCV) or for the preparation of a medicament for such treatment are set out in table 1 (see below):

Table 1

Example	Structure	Name
1		2'-Deoxy-2'-fluorocytidine
2		9-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-2,6-diaminopurine

3		2'-Deoxy-2'-fluoroguanosine
4		2'-Deoxy-2'-fluorocytidine 5'-O-triphosphate mono lithium salt

Assay Methods

The activity of 2'-deoxy-2'-fluorocytidine was determined using an adaptation of the method reported by Lohmann et al [V. Lohmann et al., Science, 1999, 285, 110-113].

HCV Replicon Assay:

The HCV replicon-containing cell line was used to demonstrate the ability of 2'-deoxy-2'-fluorocytidine to inhibit the replication of HCV replicon RNA in cells. Since the replicon RNA replication mimics the replication of the HCV RNA in infected hepatocytes, it is believed that those small molecules that have the above property are interesting for further development as anti-HCV drugs.

The inhibition of the HCV replicon RNA replication will lead to a decrease of the replicon RNA in the cell, which can be measured using a method that specifically quantifies this RNA.

The assay is based on the idea of using a reporter as a simple readout for intracellular HCV replicon RNA level. For this purpose the Renilla luciferase gene was introduced into the first open reading frame of a replicon construct NK5.1 (Krieger *et al.*, J. Virol. 75:4614), immediately after the internal ribosome entry site (IRES) sequence, and fused with the neomycin phosphotransferase (NPTII) gene via a self-cleavage peptide 2A from foot and mouth disease virus (Ryan & Drew, EMBO Vol 13:928-933). After *in vitro* transcription the RNA was electroporated into human hepatoma Huh7 cells, and G418-resistant colonies were isolated and expanded. Stably selected cell line 2209-23 was shown to contain replicative HCV subgenomic RNA, and the activity of Renilla luciferase expressed by the replicon reflects its RNA level in the cells.

For the assay procedure, Renilla Luciferase HCV replicon cells (2209-23) that cultured in Dulbecco's MEM (GibcoBRL cat no. 31966-021) with 5% fetal calf serum (FCS) (GibcoBRL cat no. 10106-169) were plated onto a 96-well plate at 5000 cells per well, and incubated overnight. Twenty-four hours later, different dilutions of chemical compounds in the growth medium were added to the cells, which were then further incubated at 37°C for three days. The assay was carried out in duplicate plates, one in opaque white and one in transparent, in order to measure the activity and cytotoxicity of a chemical compound in parallel ensuring the activity seen is not due to reduction on cell proliferation.

At the end of the incubation time, the cells in the white plate were harvested and luciferase activity was measured by using a Dual-Luciferase reporter assay system (Promega cat no. E1960). All the reagents described in the following paragraph were

included in the manufacturer's kit, and the manufacturer's instructions were followed for preparations of the reagents. Briefly, the cells were washed twice with 200µl PBS (phosphate buffered saline; pH 7.0) per well and lysed with 25µl of 1x passive lysis buffer prior to incubation at room temperature for 20 min. One hundred microlitre of LAR II reagent was added to each well. The plate was then inserted into the LB 96V microplate luminometer (MicroLumatPlus, Berthold), and 100 µl of Stop & Glo reagent was injected into each well by the machine and the signal measured using a 2-second delay, 10-second measurement program. The IC_{50}, the concentration of the drug required for reducing the replicon level by 50% in relation to the untreated cell control value, can be calculated from the plot of the percentage reduction of the luciferase activity vs. drug concentration.

For the cytotoxicity assay, WST-1 reagent from Roche Diagnostic (cat no. 1644807) was used. Ten microlitre of WST-1 reagent was added to each well including wells that contained media alone as blanks. Cells were then incubated for 1 to 1.5 hours at 37°C, and the OD value was measured by a 96-well plate reader at 450nm (reference filter at 650nm). Again CC_{50}, the concentration of the drug required for reducing cell proliferation by 50% in relation to the untreated cell control value, can be calculated from the plot of percentage reduction of the WST-1 value vs. drug concentration.

HCV NS5B polymerase assay (Hepatitis C Virus Non-Structural Protein 5B RNA-dependent RNA polymerase assay):

In order to establish the mechanism of action of 2'-deoxy-2'-fluorocytidine the activity of the 5'-O-triphosphates was measured against HCV NS5B RNA-dependent RNA polymerase enzyme. For this procedure full length NS5B polymerase bearing a C-terminal 6-histidine tag was used (V Lohmann, U Herian and R Bartenschlager J Virol, 1997, 71(11), 8416).

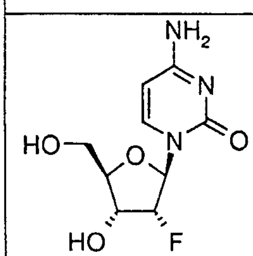
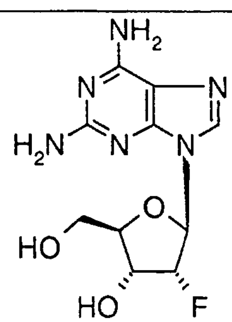
Reaction mixtures containing final concentrations of 40mM N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) at pH 8.0, 4mM dithiothreitol (DTT), 4mM magnesium acetate, Poly(rI):Oligo$(dC)_{16}$ template (0.1mg:0.01mg; annealed by heating a mixture of 5ml of 0.1mg/ml Poly(rI) and 5ml of 10mg/ml Oligo$(dC)_{16}$ to 95°C for 5 minutes and then cooling to 30°C over 20 minutes) and 500nM $[^3H]$-cytidine 5'-triphosphate ($[^3H]$-CTP; specific activity 740 GBq/mmol) (Amerham Pharmacia Biotech) in 35µl volume were incubated with 5µl aqueous solutions of nucleoside triphosphate and left for 5 minutes at room temperature. Usually ten compound dilutions were used for each IC_{50} determination. 10µl of a 5µg/ml solution of HCV NS5B polymerase was added and the mixture incubated for 2 hours at 30°C. Positive controls containing no compound and negative controls containing no enzyme were included in each assay.

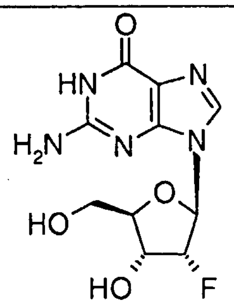
Reactions were terminated by addition of 50µl of 20% (v/v) trichloroacetic acid followed by incubation at 4°C for 30 minutes. After filtering, washing 3 times with 200µl portions of 10%(v/v) trichloroacetic acid and 3 times with 200µl portions of 70%(v/v) ethanol then drying, the reaction product was quantified by adding 25µl of scintillation cocktail (Ecoscint A purchased from National Diagnostics) followed by scintillation counting.

The concentration of compound (IC_{50}) required to reduce [^{3}H]-CTP incorporation by 50% relative to the control containing no compound was calculated from a plot of the radioactive response vs. nucleoside triphosphate concentration.

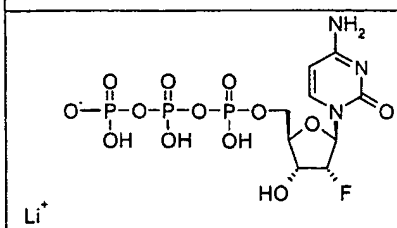
In the HCV Replicon assay, compounds of the formulas I range in activity from an IC_{50} of about 0.01 to about 100 µM, with preferred compounds having a range of activity from about 0.01 to about 50 µM, more preferably about 0.01 to 30 µM, and most preferably about 0.01 to 15 µM.

HCV Replicon assay:

Stucture	Name	IC_{50} (µM)
	2'-Deoxy-2'-fluorocytidine	0.74
	9-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-2,6-diaminopurine	10

	2'-Deoxy-2'-fluoroguanosine	62%@20

HCV NS5B RdR polymerase assay:

Stucture	Name	IC_{50} (μM)
	2'-deoxy-2'-fluorocytidine 5'-O-triphosphate mono lithium salt	1.8

The above data demonstrate, that 2'-deoxy-2'-fluoro nucleoside derivatives of formula I are inhibiting subgenomic hepatitis C virus replication in a hepatoma cell line. The mode of action has been confirmed by in vitro inhibition experiments with purified HCV NS5B polymerase and the 5'-O-triphosphate derivative of 2'-deoxy-2'-fluorocytidine. The compounds of formula I therefore have the potential to be efficacious as antiviral drugs for the treatment of HCV infections in humans, or are metabolized to compounds that exhibit such activity.

In another embodiment of the invention, the active compound or its derivative or salt can be administered in combination or alternation with another antiviral agent, such as an anti-hepatitis agent, including those of formula I. When the active compound or its derivative or salt is administered in combination or alternation with another antiviral agent its activity may be increased.

In certain pharmaceutical dosage forms, the pro-drug form of the compounds, including acylated (especially acetylated) derivatives, pyridine esters and various salt forms of the present compounds are preferred. One of ordinary skill in the art will recognise how to readily modify the present compounds to pro-drug forms to facilitate delivery of active compounds to a target site within the host organism or patient. One of ordinary skill in the art will also take advantage of favourable pharmacokinetic parameters of the pro-drug forms, where applicable, in delivering the present compounds to targeted site within the host organism or patient to maximise the intended effect of the compound.

The active compound can be administered as any derivative that upon administration to the recipient, is capable of providing directly or indirectly, the parent compound. Furthermore, the modifications can affect the biological activity of the compound, in some cases increasing the activity over the parent compound. This can easily be assessed by preparing the derivative and testing its anti-HCV activity according to the methods described herein.

The 2'-deoxy-2'-fluoro nucleoside derivatives provided by the present invention or the medicaments thereof may be used in monotherapy or combination therapy, i.e. the treatment may be in conjunction with the administration of one or more additional therapeutically active substance(s), for example, an immune system modulator such as an interferon, interleukin, tumor necrosis factor or colony stimulating factor; an antiviral agent or an anti-inflammatory agent. When the treatment is combination therapy, such administration may be concurrent or sequential with respect to that of the 2'-deoxy-2'-fluoro nucleoside derivatives of the present invention. Concurrent administration, as used herein thus includes administration of the agents at the same time or at different times.

Administration of the active compound (2'-deoxy-2'-fluoro nucleoside derivatives) provided by the present invention, as well as their pharmaceutically useable salts, can be used as medicaments in the form of any pharmaceutical formulation, e.g. oral, topical, parenteral (or intrasternal injection or infusion techniques), e.g. in the form of injection solutions, nasally, e.g. in the form of nasal sprays, or inhalation spray, topically and so forth, intramuscular, intravenous, subcutaneous, transdermal (which may include a penetration enhancement agent), buccal and suppository administration and may range from a continuous intravenous drip to several oral administrations per day (for example,

Q.I.D). Further, the pharmaceutical formulation can be administered enterally, either orally, e.g. in the form of tablets, coated tablets, dragées, hard and soft gelatine capsules, solutions, emulsions, syrups, or suspensions, or rectally, e.g. in the form of suppositories.

For the manufacture of pharmaceutical preparations, the 2'-deoxy-2'-fluoro nucleoside derivatives, as well as their pharmaceutically acceptable salts, can be formulated with a therapeutically inert, inorganic or organic excipient for the production of tablets, coated tablets, dragées, hard and soft gelatine capsules, solutions, emulsions or suspensions.

By way of example, it is contemplated that compounds according to the present invention can be formulated in admixture with a pharmaceutically acceptable carrier. For example, the compounds of the present invention can be administered orally as pharmacologically acceptable salts. Because the compounds of the present invention are mostly water soluble, they can be administered intravenously in physiological saline solution (e.g., buffered to a pH of about 7.2 to 7.5). Conventional buffers such as phosphates, bicarbonates or citrates can be used for this purpose. Of course, one of ordinary skill in the art may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising their therapeutic activity. In particular, the modification of the present compounds to render them more soluble in water or other vehicle, for example, may be easily accomplished by minor modifications (salt formulation, esterification, etc.) which are well within the ordinary skill in the art. It is also well within the ordinary skill of the art to modify the route of administration and dosage regimen of a particular compound in order to manage the pharmacokinetics of the present compounds for maximum beneficial effect in patients.

For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other ingredients including those which aid dispersion may be included. Of course, where sterile water is to be used and maintained as sterile, the compositions and carriers must also be sterilized. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

Suitable excipients for tablets, coated tablets, dragées, and hard gelatin capsules are, for example, lactose, corn starch and derivatives thereof, talc, and stearic acid or its salts.

If desired, the tablets or capsules may be enteric-coated or sustained release by standard techniques.

Suitable excipients for soft gelatine capsules are, for example, vegetable oils, waxes, fats, semi-solid and liquid polyols.

Suitable excipients for injection solutions are, for example, water, saline, alcohols, polyols, glycerine or vegetable oils.

Suitable excipients for suppositories are, for example, natural and hardened oils, waxes, fats, semi-liquid or liquid polyols.

Suitable excipients for solutions and syrups for enteral use are, for example, water, polyols, saccharose, invert sugar and glucose.

The pharmaceutical preparations of the present invention may also be provided as sustained release formulations or other appropriate formulations.

The pharmaceutical preparations can also contain preservatives, solubilizers, stabilizers, wetting agents, emulsifiers, sweeteners, colorants, flavourants, salts for adjustment of the osmotic pressure, buffers, masking agents or antioxidants.

The pharmaceutical preparations may also contain other therapeutically active agents known in the art.

The 2'-deoxy-2'-fluoro nucleoside derivatives provided by the present invention are useful in the treatment of immune mediated conditions and diseases, viral diseases, bacterial diseases, parasitic diseases, inflammatory diseases, hyperproliferative vascular diseases, allograft rejection, tumours, and cancers.

The dosage can vary within wide limits and will, of course, be adjusted to the individual requirements in each particular case. For oral administration, a daily dosage of between about 0.01 and about 1000 mg/kg body weight per day should be appropriate in monotherapy and/or in combination therapy. A preferred daily dosage is between about 0.1 and about 500 mg/kg body weight, more preferred 0.1 and about 100 mg/kg body weight and most preferred 1.0 and about 100 mg/kg body weight per day. A typical preparation will contain from about 5% to about 95% active compound (w/w) . The daily dosage can be administered as a single dosage or in divided dosages, typically between 1 and 5 dosages per day.

It will be understood that references herein to treatment extend to prophylaxis as well as to the treatment of existing conditions, and that the treatment of animals includes the treatment of humans as well as other mammals. Furthermore, the term "treatment of a hepatitis C virus (HCV) infection", as used herein, includes the treatment or prophylaxis

of a disease or a condition associated with or mediated by hepatitis C virus (HCV) infection, or the clinical symptoms thereof.

In the present specification "comprise" means "includes or consists of" and "comprising" means "including or consisting of".

The features disclosed in the foregoing description, or the following claims, or the accompanying drawings, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for attaining the disclosed result, as appropriate, may, separately, or in any combination of such features, be utilised for realising the invention in diverse forms thereof.

The compounds of the present invention are known in the art and can be prepared by known methods, especially as described below:

Example 1

2'-Deoxy-2'-fluorocytidine can be purchased from Sigma-Aldrich Company Ltd., Cat. No. F8883 or prepared by methods known to the art for example from 2,2'-O-anhydrocytidine as described by R Mengel and W Guschlbauer Angew Chemie Intl Ed 1978, 17, 525.

Example 2

9-(2-Deoxy-2-fluoro-β-D-ribofuranosyl)-2,6-diaminopurine can be prepared by the method of H. J. Thomas et al, Nucleosides and Nucleotides, 1994, 13, 309.

Example 3

2'-Deoxy-2'-fluoroguanosine can be prepared by the method of B. S. Ross et al, Nucleosides and Nucleotides, 1997, 16, 1645.

Example 4

The 5'-O-triphosphate derivative of 2'-deoxy-2'-fluorocytidine can be purchased from Trilink BioTechnologies Inc., Cat. No. N-1008-1 or prepared by methods known to the art for example as described by K Burgess and D Cook Chemical Reviews 2000, 100, 2047.

Methods for the monophosphorylation of organic compounds including nucleosides have been reviewed by L A Slotin, Synthesis, 1977, 737. More recently other nucleoside phosphorylation procedures have been described: M Uchiyama et al J. Org. Chem., 1993, 58,373; R Caputo et al, Synlett., 1997, 739 and M Taktakishvili and V Nair Tet. Lett. 2000, 41, 7173. Other procedures for monophosphorylation that may be useful for nucleosides are described by C E McKenna and J Schmidhauser, J.Chem.Soc.,Chem.Commun.,1979, 739 and J K Stowell and T S Widlanski Tet. Lett., 1995, 1825. Synthesis of di and triphosphate derivatives are reviewed in K H Scheit, Nucleotide Analogues, 1980, Wiley Interscience and by K Burgess and D Cook Chemical Reviews, 2000, 100, 2047.

The compounds represented by formula I may be prepared by any of the methods known in the art for the preparation of similar 2'-fluoronucleoside derivatives. E.g see P Herdewijn et al Nucleosides and Nucleotides, 1989, 8, 65 or H Hayakawa et al Chem Pharm Bull, 1990, 38, 1136 and in particular R Mengel and W Guschlbauer Angew Chemie Intl Ed 1978, 17, 525 or H J Thomas et al, Nucleosides and Nucleotides 1994, 13, 309 or B S Ross, et al, Nucleosides and Nucleotides, 1997, 16, 1645.

Such methods may be adapted for the synthesis of the alternative stereoisomers represented by formula I for example L-nucleosides. The general synthesis of L-nucleosides has been described (P Wang et al, Antiviral Research, 1998, 40, 19; E Moyroud and P Strazewski Tetrahedron, 1999, 55, 1277). Introduction of a 2'-fluoro substituent can be accomplished using the methods described for the corresponding D-nucleoside analogues in the references above.

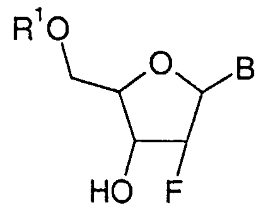
Where synthesis of the compound of formula I employs a condensation reaction of a purine or pyrimidine base with a suitably protected 2-fluoro-furanose derivative such as that described by H J Thomas et al Nucleosides and Nucleotides, 1994, 13, 309, then mixtures of anomeric nucleoside derivatives will often result. The α and β-nucleosides can

be separated by standard techniques known to the art such as recrystallisation, column chromatography, high performance liquid chromatography or super critical fluid chromatography.

Further information for the preparation of compounds of formula I or I-a can be deduced from the following references: WO 99/43691, WO 98/16184, C. R.Wagner et al Medicinal Research Reviews, 2000, 20(6), 417 or R. Jones and N. Bischofberger, Antiviral Research 1995, 27, 1).

Claims

1. Use of compounds of formula I

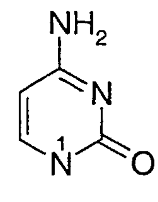


I

wherein

R^1 is hydrogen or phosphate and

B signifies a 1-pyrimidinyl or 9-purinyl residue of formulae B1, B2 or B3



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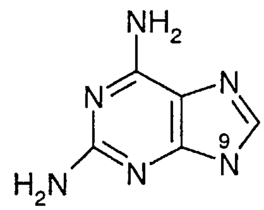

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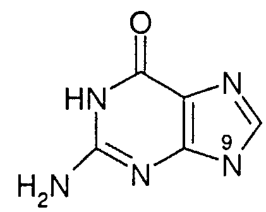
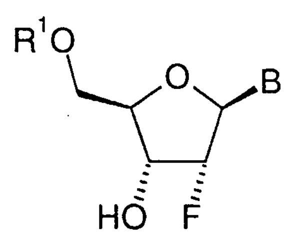


B3

and of pharmaceutically acceptable salts thereof for the treatment of diseases mediated by the hepatitis C virus (HCV) or for the preparation of medicaments for such treatment.

2. Use of compounds as claimed in claim 1 wherein the compounds are β-D or β-L ribofuranosides and pharmaceutically acceptable salts thereof.

3. Use of compounds as claimed in claims 1 or 2 of formula I-a



I-a

wherein R^1 and B are as defined in claim 1,

and of pharmaceutically acceptable salts thereof.

4. Use of compounds of formula I or I-a as claimed in any one of claims 1 to 3 wherein

R^1 is as defined above and B signifies 1-pyrimidinyl,

and of pharmaceutically acceptable salts thereof.

5. Use of a compound of formula I or I-a as claimed in any one of claims 1 to 3 which compound is

2'-deoxy-2'-fluorocytidine,

9-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-2,6-diaminopurine,

2'-deoxy-2'-fluoroguanosine, or

2'-deoxy-2'-fluorocytidine 5'-O-triphosphate mono lithium salt.

6. A compound as defined in any one of claims 1 to 5 or a pharmaceutically acceptable salt thereof for the treatment of diseases mediated by the hepatitis C virus (HCV) or for the preparation of medicaments for such treatment.

7. A pharmaceutical composition on the basis of a pharmaceutically effective amount of a compound of formula I or I-a or a pharmaceutically acceptable salt thereof, as defined in any one of claims 1 to 5 for the treatment of diseases mediated by the hepatitis C virus (HCV) or for the preparation of a medicament for such treatment.

8. The use of a pharmaceutical composition on the basis of a pharmaceutically effective amount of a compound of formula I or I-a or a pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 5 for the treatment of diseases mediated by the hepatitis C virus (HCV).

9. The invention as hereinbefore described.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/05340

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/708 A61K31/7076 A61K31/7068 A61P31/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data, PAJ, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 43691 A (CHOI YONGSEOK ;CHU CHUNG K (US); HONG JOON H (US); SHI JUNXING (US) 2 September 1999 (1999-09-02) cited in the application page 8, line 26 -page 10, line 9 page 11, line 9 - line 13 page 12, line 30 -page 13, line 14 page 13, line 30 -page 14, line 17 page 15, line 9 -page 17, line 11 page 28, line 9 - line 18 page 31, line 6 - line 20 page 35, line 15 -page 37, line 25 page 79, line 14 - line 25	1-9
X	the whole document ---	1-9

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 September 2002	25/09/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Economou, D

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 02/05340

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	TIANWEI MA ET AL.: "Structure-Activity Relationships of 1-(2-Deoxy-2-fluoro-beta-L-arabinofuranosyl)pyrimidine Nucleosides as Anti-Hapatitis B Virus Agents" J.MED.CHEM., vol. 39, no. 14, 1996, pages 2835-2843, XP002212830 page 2836; example 27 page 2841, right-hand column, line 36 - line 41 ---	6,7,9
X	TIANWEI MA ET AL.: "Synthesis and Anti-Hepatitis B Virus Activity of 9-(2-deoxy-2-fluoro-beta-L-arabinofuranosyl)purine Nucleosides" J.MED.CHEM., vol. 40, no. 17, 1997, pages 2750-2754, XP002212831 compounds 22 and 23 table 1 page 2753, left-hand column, line 33 - line 58 page 2753, right-hand column, line 6 - line 23 ---	6,7,9
X	US 5 587 362 A (CHU CHUNG K ET AL) 24 December 1996 (1996-12-24) L-FC figure 1 ---	6,7,9
X	US 5 968 826 A (COWSERT LEX M ET AL) 19 October 1999 (1999-10-19) column 18, line 6 - line 30 ---	6,7,9
X,P	WO 02 32920 A (PHARMASSET LTD ;STUYVER LIEVEN (US); WATANABE KYOICHI A (US)) 25 April 2002 (2002-04-25) compound DB=2'-deoxy-2'-fluoroguanosine page 53; table 2 page 16, line 1 - line 10 -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 02/05340

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
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			CA	2322008	A1	02-09-1999
			CN	1332747	T	23-01-2002
			EP	1058686	A1	13-12-2000
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			WO	9943691	A1	02-09-1999
			US	6348587	B1	19-02-2002
US 5587362	A	24-12-1996	AU	710262	B2	16-09-1999
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			WO	9520595	A1	03-08-1995
			US	5565438	A	15-10-1996
			US	5567688	A	22-10-1996
US 5968826	A	19-10-1999	AU	5570399	A	26-04-2000
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			US	6258790	B1	10-07-2001
WO 0232920	A	25-04-2002	AU	2874902	A	29-04-2002
			AU	2888502	A	29-04-2002
			WO	0232920	A2	25-04-2002
			WO	0233128	A2	25-04-2002

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 December 2002 (19.12.2002)

PCT

(10) International Publication Number
WO 02/100415 A2

(51) International Patent Classification[7]: **A61K 31/7068**, 31/7072, 31/7076, 31/708, C07H 19/06, 19/16, A61P 31/14

(21) International Application Number: PCT/EP02/06256

(22) International Filing Date: 7 June 2002 (07.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0114286.8 12 June 2001 (12.06.2001) GB

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(74) Agent: **RAUBER, Beat**; 124 Grenzacherstrasse, CH-4070 Basle (CH).

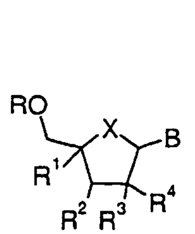
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

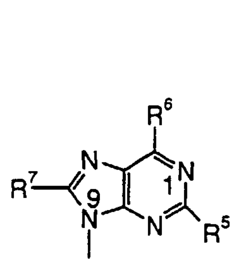
Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: 4'-SUBSTITUTED NUCLEOSIDES



(I)



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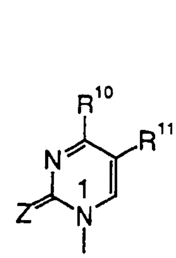


(B2)

(57) Abstract: The present invention relates to the use of nucleoside derivatives of Formula (I) wherein B signifies a 9-purinyl residue B1 of Formula (B1) or a 1-pyrimidyl residue B2 of Formula (B2) wherein the symbols are as defined in the specification, and of pharmaceutically acceptable salts thereof; for the treatment of diseases mediated by the Hepatitis C Virus (HCV), for the preparation of a medicament for such treatment and to pharmaceutical compositions containing such compounds.

WO 02/100415 A2

4'-Substituted Nucleosides

The invention relates to nucleoside derivatives as inhibitors of HCV replicon RNA replication. In particular, the invention is concerned with the use of purine and pyrimidine nucleoside derivatives as inhibitors of subgenomic Hepatitis C Virus (HCV) RNA replication and pharmaceutical compositions containing such compounds.

Hepatitis C virus is the leading cause of chronic liver disease throughout the world. Patients infected with HCV are at risk of developing cirrhosis of the liver and subsequent hepatocellular carcinoma and hence HCV is the major indication for liver transplantation. Only two approved therapies are currently available for the treatment of HCV infection (R. G. Gish, Sem. Liver. Dis., 1999, 19, 35). These are interferon-α monotherapy and, more recently, combination therapy of the nucleoside analogue, ribavirin (Virazole), with interferon-α.

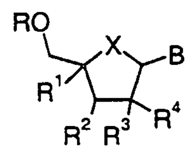
Many of the drugs approved for the treatment of viral infections are nucleosides or nucleoside analogues and most of these nucleoside analogue drugs inhibit viral replication, following conversion to the corresponding triphosphates, through inhibition of the viral polymerase enzymes. This conversion to the triphosphate is commonly mediated by cellular kinases and therefore the direct evaluation of nucleosides as inhibitors of HCV replication is only conveniently carried out using a cell-based assay. For HCV the availability of a true cell-based viral replication assay or animal model of infection is lacking.

Hepatitis C virus belongs to the family of Flaviridae. It is an RNA virus, the RNA genome encoding a large polyprotein which after processing produces the necessary replication machinery to ensure synthesis of progeny RNA. It is believed that most of the non-structural proteins encoded by the HCV RNA genome are involved in RNA replication. Lohmann et al. [V. Lohmann et al., Science, 1999, 285,

110-113] have described the construction of a Human Hepatoma (Huh7) cell line in which subgenomic HCV RNA molecules have been introduced and shown to replicate with high efficiency. It is believed that the mechanism of RNA replication in these cell lines is identical to the replication of the full length HCV RNA genome in infected hepatocytes. The subgenomic HCV cDNA clones used for the isolation of these cell lines have formed the basis for the development of a cell-based assay for identifying nucleoside analogue inhibitors of HCV replication.

The compounds of formula I have been shown to be inhibitors of subgenomic Hepatitis C Virus replication in a hepatoma cell line. These compounds have the potential to be efficacious as antiviral drugs for the treatment of HCV infections in human.

The invention is concerned with the use of compounds of the formula I

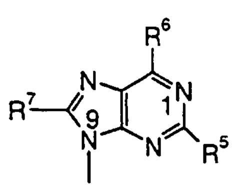


I

wherein

R	is hydrogen or $-[P(O)(OH)-O]_nH$ and n is 1, 2 or 3;
R^1	is alkyl, alkenyl, alkynyl, haloalkyl, alkylcarbonyl, alkoxycarbonyl, hydroxyalkyl, alkoxyalkyl, alkoxy, cyano, azido, hydroxyiminomethyl, alkoxyiminomethyl, halogen, alkylcarbonylamino, alkylaminocarbonyl, azidoalkyl, aminomethyl, alkylaminomethyl, dialkylaminomethyl or heterocyclyl;
R^2	is hydrogen, hydroxy, amino, alkyl, hydroxyalkyl, alkoxy, halogen, cyano, or azido;
R^3 and R^4	are hydrogen, hydroxy, alkoxy, halogen or hydroxyalkyl, provided that at least one of R^3 and R^4 is hydrogen; or
R^3 and R^4	together represent $=CH_2$ or $=N-OH$, or
R^3 and R^4	both represent fluorine;
X	is O, S or CH_2;

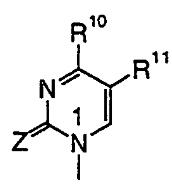
B signifies a 9-purinyl residue B1 of formula



B1

wherein

R^5	is hydrogen, hydroxy, alkyl, alkoxy, alkylthio, NHR^8, halogen or SH;
R^6	is hydroxy, NHR^8, $NHOR^9$, $NHNR^8$, $-NHC(O)OR^{9'}$ or SH;
R^7	is hydrogen, hydroxy, alkyl, alkoxy, alkylthio, NHR^8, halogen, SH or cyano;
R^8	is hydrogen, alkyl, hydroxyalkyl, arylcarbonyl or alkylcarbonyl;
R^9	is hydrogen or alkyl;
$R^{9'}$	is alkyl; and
B	signifies a 1-pyrimidyl residue B2 of formula



B2

wherein

Z	is O or S;
R^{10}	is hydroxy, NHR^8, $NHOR^9$, $NHNR^8$, $-NHC(O)OR^{9'}$ or SH;
R^{11}	is hydrogen, alkyl, hydroxy, hydroxyalkyl, alkoxyalkyl, haloalkyl or halogen;
R^8 R^9 and $R^{9'}$	are as defined above;

and of pharmaceutically acceptable salts thereof;

for the treatment of diseases mediated by the Hepatitis C Virus (HCV) or for the preparation of medicaments for such treatment.

In compounds, wherein R is a phosphate group $-[P(O)(OH)-O]_nH$, n is preferably 1. The phosphate group may be in the form of a stabilized monophosphate prodrug or other pharmaceutically acceptable leaving group which when administered in vivo, is capable of providing a compound wherein R is monophosphate. These "pronucleotides" can improve the properties such as activity, bioavailability or stability of the parent nucleotide.

Examples of substituent groups which can replace one or more of the hydrogens in the phosphate moiety are described in C. R.Wagner et al., Medicinal Research Reviews, 2000, 20(6), 417 or in R. Jones and N. Bischofberger, Antiviral Research 1995, 27, 1. Such pronucleotides include alkyl and aryl phosphodiesters, steroid phosphodiesters, alkyl and aryl phosphotriesters, cyclic alkyl phosphotriesters, cyclosaligenyl (CycloSal) phosphotriesters, S-acyl-2-thioethyl

(SATE) derivatives, dithioethyl (DTE) derivatives, pivaloyloxymethyl phosphoesters, para-acyloxybenzyl (PAOB) phosphoesters, glycerolipid phosphodiesters, glycosyl lipid phosphotriesters, dinucleosidyl phosphodiesters, dinucleoside phosphotriesters, phosphorodiamidates, cyclic phosphoramidates, phosphoramidate monoesters and phosphoramidate diesters.

The invention also includes pro-drugs or bioprecursors of the parent nucleoside which are converted *in vivo* to the compound of formula I wherein R is hydrogen, or at least one of R^2, R^3 and R^4 is hydroxy. Preferred pro-drug derivatives include carboxylic esters in which the non-carbonyl moiety of the ester group is selected from straight or branched alkyl (e.g. methyl, n-propyl, n-butyl or tert.-butyl), alkoxyalkyl (e.g. methoxymethyl), aralkyl (e.g. benzyl), aryloxyalkyl (e.g. phenoxymethyl), aryl (e.g. phenyl optionally substituted by halogen, C_{1-4} alkyl or C_{1-4} alkoxy or amino); sulphonate esters such as alkylsulphonyl or arylsulphonyl (e.g. methanesulphonyl); amino acid esters (e.g.L-valyl or L-isoleucyl) or pharmaceutically acceptable salts thereof. The preparation is carried out according to known methods in the art, for example methods known from textbooks on organic chemistry (e.g. from J. March (1992), "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure", 4th ed. John Wiley & Sons).

The term "alkyl" as used herein denotes a straight or branched chain hydrocarbon residue containing 1 to 12 carbon atoms. Preferably, the term "alkyl" denotes a straight or branched chain hydrocarbon residue containing 1 to 7 carbon atoms. Most preferred are methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, tert.-butyl or pentyl. The alkyl may be unsubstituted or substituted. The substituents are selected from one or more of cycloalkyl, nitro, amino, alkyl amino, dialkyl amino, alkyl carbonyl and cycloalkyl carbonyl.

The term "cycloalkyl" as used herein denotes an optionally substituted cycloalkyl group containing 3 to 7 carbon atoms, e. g. cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl.

The term "alkoxy" as used herein denotes an optionally substituted straight or branched chain alkyl-oxy group wherein the "alkyl" portion is as defined above such as methoxy, ethoxy, n-propyloxy, i-propyloxy, n-butyloxy, i-butyloxy, tert.-butyloxy, pentyloxy, hexyloxy, heptyloxy including their isomers.

The term "alkoxyalkyl" as used herein denotes an alkoxy group as defined above which is bonded to an alkyl group as defined above. Examples are

methoxymethyl, methoxyethyl, methoxypropyl, ethoxymethyl, ethoxyethyl, ethoxypropyl, propyloxypropyl, methoxybutyl, ethoxybutyl, propyloxybutyl, butyloxybutyl, tert. -butyloxybutyl, methoxypentyl, ethoxypentyl, propyloxypentyl including their isomers.

The term "alkenyl" as used herein denotes an unsubstituted or substituted hydrocarbon chain radical having from 2 to 7 carbon atoms, preferably from 2 to 4 carbon atoms, and having one or two olefinic double bonds, preferably one olefinic double bond. Examples are vinyl, 1-propenyl, 2-propenyl (allyl) or 2-butenyl (crotyl).

The term "alkynyl" as used herein denotes to unsubstituted or substituted hydrocarbon chain radical having from 2 to 7 carbon atoms, preferably 2 to 4 carbon atoms, and having one or where possible two triple bonds, preferably one triple bond. Examples are ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl or 3-butynyl.

The term "hydroxyalkyl" as used herein denotes a straight or branched chain alkyl group as defined above wherein 1, 2, 3 or more hydrogen atoms are substituted by a hydroxy group. Examples are hydroxymethyl, 1-hydroxyethyl, 2-hydroxyethyl, 1-hydroxypropyl, 2-hydroxypropyl, 3-hydroxypropyl, hydroxyisopropyl, hydroxybutyl and the like.

The term "haloalkyl" as used herein denotes a straight or branched chain alkyl group as defined above wherein 1, 2, 3 or more hydrogen atoms are substituted by a halogen. Examples are 1-fluoromethyl, 1-chloromethyl, 1-bromomethyl, 1-iodomethyl, trifluoromethyl, trichloromethyl, tribromomethyl, triiodomethyl, 1-fluoroethyl, 1-chloroethyl, 1-bromoethyl, 1-iodoethyl, 2-fluoroethyl, 2-chloroethyl, 2-bromoethyl, 2-iodoethyl, 2,2-dichloroethyl, 3-bromopropyl or 2,2,2-trifluoroethyl and the like.

The term "alkylthio" as used herein denotes a straight or branched chain (alkyl)S- group wherein the "alkyl" portion is as defined above. Examples are methylthio, ethylthio, n-propylthio, i-propylthio, n-butylthio, i-butylthio or tert.-butylthio.

The term "aryl" as used herein denotes an optionally substituted phenyl and naphthyl (e. g. 1-naphthyl, 2-naphthyl or 3-naphthyl). Suitable substituents for aryl can be selected from those named for alkyl, in addition however, halogen, hydroxy

and optionally substituted alkyl, haloalkyl, alkenyl, alkynyl and aryloxy are substituents which can be added to the selection.

The term "heterocyclyl" as used herein denotes an optionally substituted saturated, partially unsaturated or aromatic monocyclic, bicyclic or tricyclic heterocyclic systems which contain one or more hetero atoms selected from nitrogen, oxygen and sulfur which can also be fused to an optionally substituted saturated, partially unsaturated or aromatic monocyclic carbocycle or heterocycle.

Examples of suitable heterocycles are oxazolyl, isoxazolyl, furyl, tetrahydrofuryl, 1,3-dioxolanyl, dihydropyranyl, 2-thienyl, 3-thienyl, pyrazinyl, isothiazolyl, dihydrooxazolyl, pyrimidinyl, tetrazolyl, 1-pyrrolidinyl, 2-pyrrolidinyl, 3-pyrrolidinyl, pyrrolidinonyl, (N-oxide)-pyridinyl, 1-pyrrolyl, 2-pyrrolyl, triazolyl e. g. 1,2,3-triazolyl or 1,2,4-triazolyl, 1-pyrazolyl, 2-pyrazolyl, 4-pyrazolyl, piperidinyl, morpholinyl (e. g. 4-morpholinyl), thiomorpholinyl (e. g. 4-thiomorpholinyl), thiazolyl, pyridinyl, dihydrothiazolyl, imidazolidinyl, pyrazolinyl, piperazinyl, 1-imidazolyl, 2-imidazolyl, 4-imidazolyl, thiadiazolyl e. g. 1,2,3-thiadiazolyl, 4-methylpiperazinyl, 4-hydroxypiperidin-1-yl.

Suitable substituents for heterocyclyl can be selected from those named for alkyl, in addition however, optionally substituted alkyl, alkenyl, alkynyl, an oxo group (=O) or aminosulphonyl are substituents which can be added to the selection.

The term "acyl" ("alkylcarbonyl")as used herein denotes a group of formula C(=O)R wherein R is hydrogen, an unsubstituted or substituted straight or branched chain hydrocarbon residue containing 1 to 7 carbon atoms or a phenyl group. Most preferred acyl groups are those wherein R is hydrogen, an unsubstituted straight chain or branched hydrocarbon residue containing 1 to 4 carbon atoms or a phenyl group.

The term halogen stands for fluorine, chlorine, bromine or iodine, preferable fluorine, chlorine, bromine.

Within the invention the term "X" represents O, S or CH_2, preferably O or CH_2. Most preferred "X" represents O.

Within the invention the term "Z" represents O or S, preferably O.

In the pictorial representation of the compounds given throughout this application, a thickened tapered line ( ) indicates a substituent which is above

the plane of the ring to which the asymmetric carbon belongs and a dotted line ('''''') indicates a substituent which is below the plane of the ring to which the asymmetric carbon belongs.

Compounds of formula I exhibit stereoisomerism. These compounds can be any isomer of the compound of formula I or mixtures of these isomers. The compounds and intermediates of the present invention having one or more asymmetric carbon atoms may be obtained as racemic mixtures of stereoisomers which can be resolved.

Compounds of formula I exhibit tautomerism that means that the compounds of this invention can exist as two or more chemical compounds that are capable of facile interconversion. In many cases it merely means the exchange of a hydrogen atom between two other atoms, to either of which it forms a covalent bond. Tautomeric compounds exist in a mobile equilibrium with each other, so that attempts to prepare the separate substances usually result in the formation of a mixture that shows all the chemical and physical properties to be expected on the basis of the structures of the components.

The most common type of tautomerism is that involving carbonyl, or keto, compounds and unsaturated hydroxyl compounds, or enols. The structural change is the shift of a hydrogen atom between atoms of carbon and oxygen, with the rearrangement of bonds. For example, in many aliphatic aldehydes and ketones, such as acetaldehyde, the keto form is the predominant one; in phenols, the enol form is the major component.

Compounds of formula I which are basic can form pharmaceutically acceptable salts with inorganic acids such as hydrohalic acids (e.g. hydrochloric acid and hydrobromic acid), sulphuric acid, nitric acid and phosphoric acid, and the like, and with organic acids (e.g. with acetic acid, tartaric acid, succinic acid, fumaric acid, maleic acid, malic acid, salicylic acid, citric acid, methanesulphonic acid and p-toluene sulphonic acid, and the like). The formation and isolation of such salts can be carried out according to methods known in the art.

Preferred is the use of compounds of formula I, wherein

R is hydrogen;

R^1 is alkyl, alkenyl, alkynyl, haloalkyl, alkylcarbonyl, alkoxy, hydroxymethyl, cyano, azido, alkoxyiminomethyl,

alkylcarbonylamino, alkylaminomethyl or dialkyl-aminomethyl;

R^2 is hydrogen, hydroxy, alkoxy or halogen;

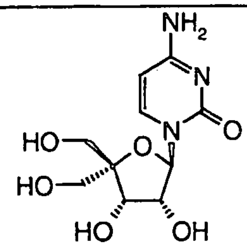
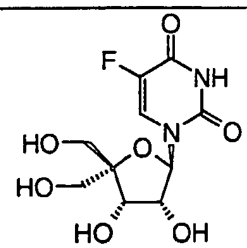
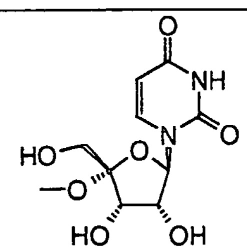
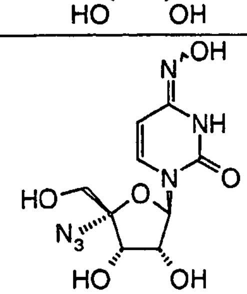
R^3 and R^4 are hydrogen, hydroxy, alkoxy, halogen or hydroxyalkyl, provided that at least one of R^3 and R^4 is hydrogen; or

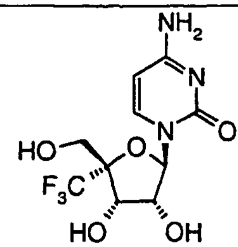
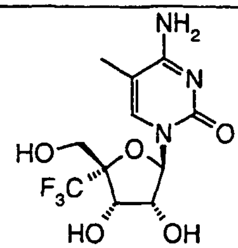
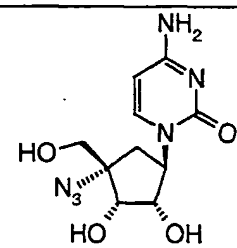
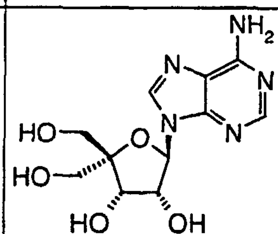
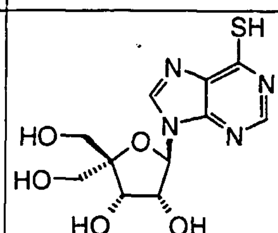
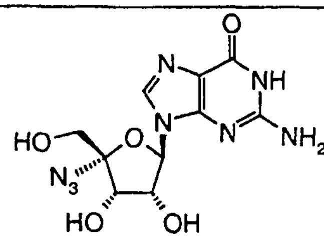
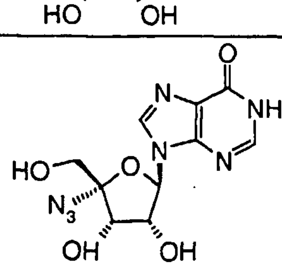
R^3 and R^4 represent fluorine;

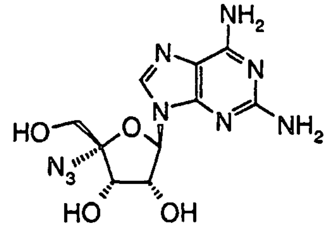
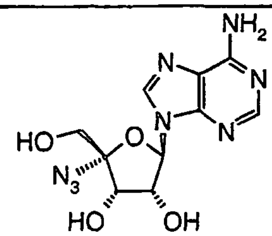
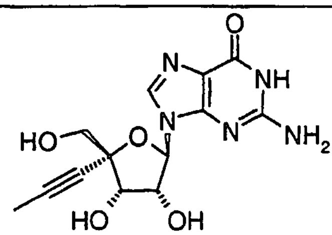
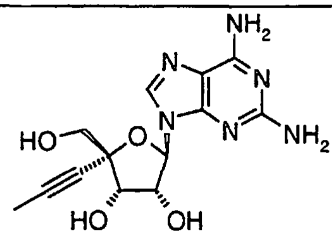
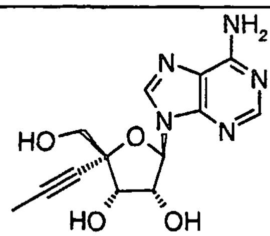
X is O or CH_2; and

B signifies a 9-purinyl residue B1 or a 1-pyrimidyl residue B2 as defined above.

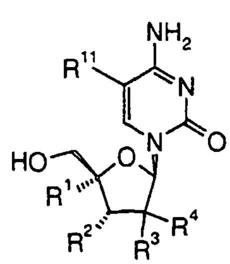
Examples of preferred compounds are listed below

Compound no.	STRUCTURE	Name
compound 6		4'-C-(Hydroxymethyl)cytidine
compound 7		5-Fluoro-4'-C-(hydroxymethyl)-uridine
compound 8		4'-C-Methoxyuridine
compound 10		(E and/or Z)-4'-C-Azidouridine 4-oxime

compound 11		4'-C-(Trifluoromethyl)cytidine
compound 12		4'-C-(Trifluoromethyl)-5-methyl-cytidine
compound 13		1-[4(S)-Azido-2(S),3(R)-dihydroxy-4-(hydroxymethyl)-1(R)-cyclopentyl]cytosine
compound 14		4'-C-(Hydroxymethyl)adenosine
compound 15		9-[4-C-(Hydroxymethyl)-beta-D-ribofuranosyl]-6-mercaptopurine
compound 16		4'-C-Azidoguanosine
compound 16-1		4'-C-Azidoinosine (9-(5-Azido-3,4-dihydroxy-5-hydroxymethyl-tetrahydro-furan-2-yl)-1,9-dihydro-purin-6-one)

compound 17		2-Amino-4'-C-Azidoadenosine
compound 18		4'-C-Azidoadenosine
compound 19		4'-C-(1-Propynyl)guanosine
compound 20		2-Amino-4'-C-(1-propynyl)-adenosine
compound 21		4'-C-(1-Propynyl)adenosine

An especially preferred group of compounds for the treatment of HCV are those of formula I-a



I-a

wherein

R^1 is alkyl, alkenyl, alkynyl, haloalkyl, alkylcarbonyl, alkoxy, hydroxymethyl, cyano, azido, alkoxyiminomethyl, alkylcarbonylamino, alkylaminomethyl or dialkyl-aminomethyl;

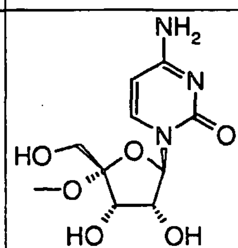
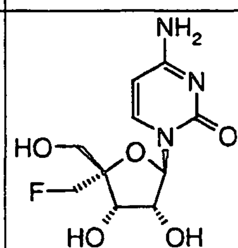
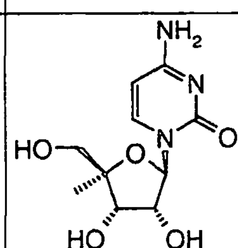
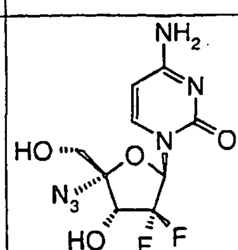
R^2 is hydrogen, hydroxy, alkoxy, or halogen;

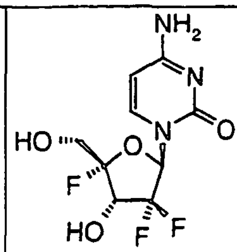
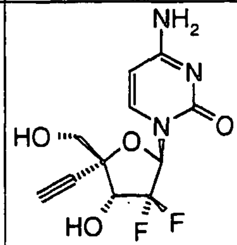
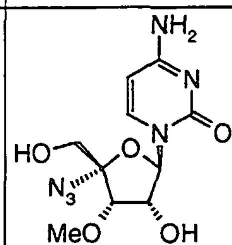
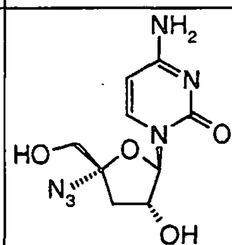
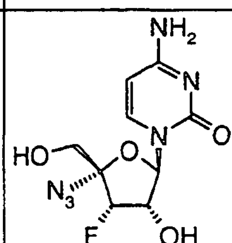
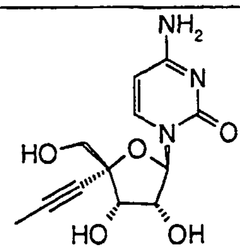
R^3 and R^4 are hydrogen, hydroxy, alkoxy, halogen or hydroxyalkyl, provided that at least one of R^3 and R^4 is hydrogen; or

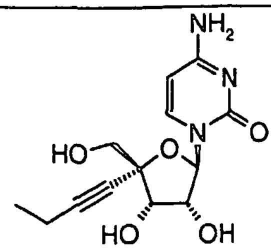
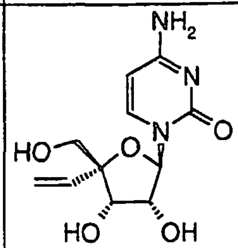
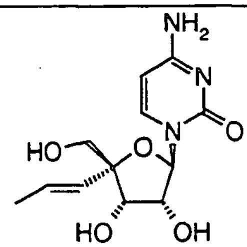
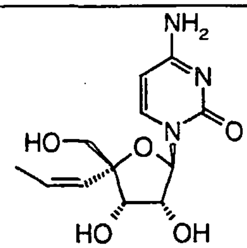
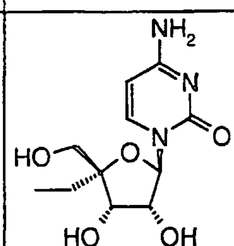
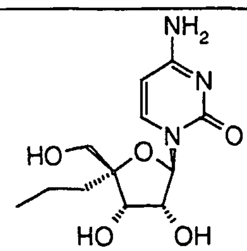
R^3 and R^4 represent fluorine.

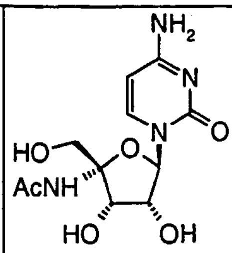
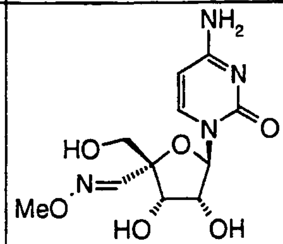
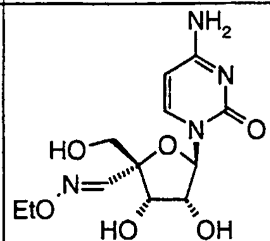
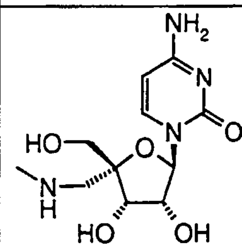
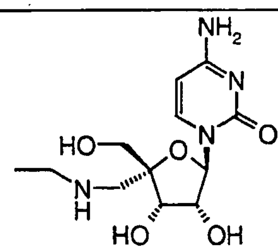
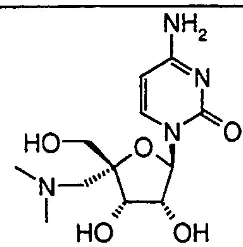
and pharmaceutically acceptable salts.

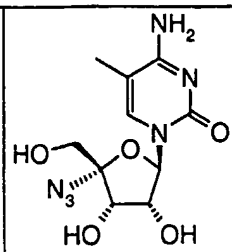
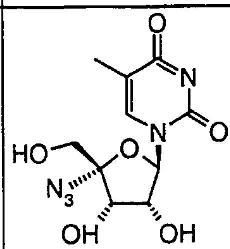
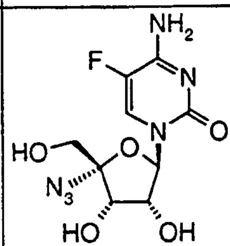
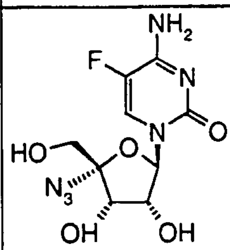
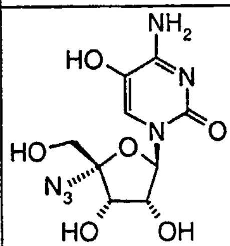
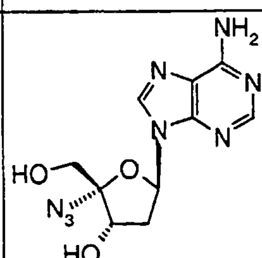
Examples of such especially preferred compounds are listed below

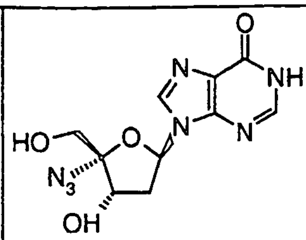
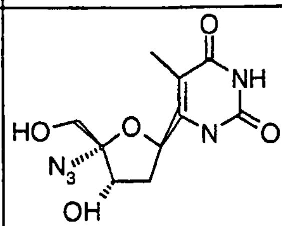
Compound no.	Structure	Name
compound 9		4'-C-Methoxycytidine
compound 22		4'-C-(Fluoromethyl)cytidine
compound 23		4'-C-Methylcytidine
compound 24		4'-C-Azido-2'-deoxy-2',2'-difluorocytidine

compound 25		2'-Deoxy-4'-C-fluoro-2',2'-difluorocytidine
compound 26		2'-Deoxy-4'-C-ethynyl-2',2'-difluorocytidine
compound 27		4'-C-Azido-3'-O-methylcytidine
compound 28		4'-C-Azido-3'-deoxycytidine
compound 29		4'-C-Azido-3'-deoxy-3'-fluorocytidine
compound 30		4'-C-(1-Propynyl)cytidine

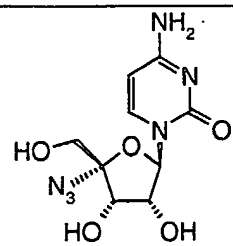
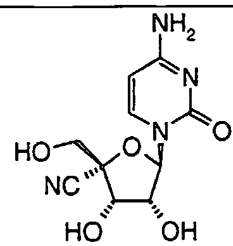
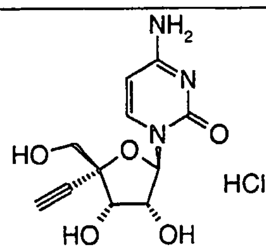
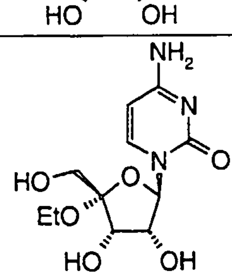
compound 31		4'-C-(1-Butynyl)cytidine
compound 32		4'-C-Vinylcytidine
compound 33		(E)-4'-C-(1-Propenyl)cytidine
compound 34		(Z)-4'-C-(1-Propenyl)cytidine
compound 35		4'-C-Ethylcytidine
compound 36		4'-C-Propylcytidine

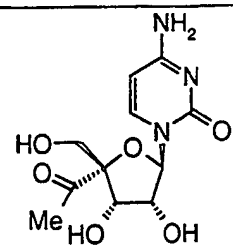
compound 37		4'-C-Acetamidocytidine
compound 38		(E)-4'-C-(Methoxyimino)cytidine
compound 39		(E)-4'-C-(Ethoxyimino)cytidine
compound 40		4'-C-[(Methylamino)methyl]-cytidine
compound 41		4'-C-[(Ethylamino)methyl]cytidine
compound 42		4'-C-[(Dimethylamino)methyl]-cytidine

compound 43		4'-C-Azido-5-methylcytidine
compound 43-1		4'-C-Azido-5-methyluridine
compound 44		4'-C-Azido-5-fluorocytidine
compound 44-1		4'-C-Azido-5-fluorouridine
compound 45		4'-C-Azido-5-hydroxycytidine
compound 46		4'-Azido-2'-deoxyadenosine

compound 47		4'-C-Azido-2'-deoxy inosine
compound 48		4'-C-Azido- 5-methyluridine

Most preferred compounds for the treatment of HCV are listed below:

Compound no.	Structure	Name
compound 1 (Example 1)		4'-C-Azidocytidine
compound 2 (Example 2)		4'-C-Cyanocytidine
compound 3 (Example 3)		4'-C-Ethynylcytidine hydrochloride (1:1)
compound 4		4'-C-Ethoxycytidine

compound 5		4'-C-Acetylcytidine

The compounds of formula I may be prepared by various methods known in the art of organic chemistry in general and nucleoside analogue synthesis in particular. The starting materials for the syntheses are either readily available from commercial sources or are known or may themselves be prepared by techniques known in the art. General reviews of the preparation of nucleoside analogues are included in the following publications:

A M Michelson "The Chemistry of Nucleosides and Nucleotides", Academic Press, New York 1963.

L Goodman "Basic Principles in Nucleic Acid Chemistry" Ed P O P Ts'O, Academic Press, New York 1974, Vol. 1, chapter 2.

"Synthetic Procedures in Nucleic acid Chemistry" Ed W W Zorbach and R S Tipson, Wiley, New York, 1973, Vol. 1 and 2.

The synthesis of carbocylic nucleosides has been reviewed by L Agrofoglio *et al*, Tetrahedron, 1994, 50, 10611.

The strategies available for the synthesis of compounds of formula I include:

1. modification or interconversion of performed nucleosides; or

2. construction of the heterocyclic base after glycosylation; or

3. condensation of a protected furanose, thiofuranose or cyclopentane derivative with a pyrimidine (B2) or purine (B1) base.

These methods will be further discussed below:

1. Modification or inter-conversion of preformed nucleosides.

Such methods include on the one hand modification of the 9-purinyl or 1-pyrimidyl residue or on the other hand modification of the carbohydrate moiety.

A. Modification of the purinyl or pyrimidyl moiety:

a) The deamination of aminopurine or aminopyrimidine nucleosides as described by J. R. Tittensor and R. T. Walker European Polymer J., 1968, 4, 39 and H. Hayatsu, Progress in Nucleic Acid Research and Molecular Biology 1976, Vol. 16, p75.

b) The conversion of the 4-hydroxy group of 4-hydroxypyrimidine nucleosides to a leaving group and displacement with nucleophilic reagents. Such leaving groups include halogen as described by J. Brokes and J. Beranek, Col. Czech. Chem. Comm., 1974, 39, 3100 or 1,2,4-triazole as described by K. J. Divakar and C. B. Reece, J. Chem. Soc. Perkin Trans. I, 1982, 1171.

c) 5-Substitution of pyrimidine nucleosides has been achieved by the use of 5-metallo derivatives such as 5-mercuri or 5-palladium for example as described by D. E. Bergstrom and J. L. Ruth J. Amer. Chem. Soc., 1976, 98, 1587. Introduction of fluoro into the 5 position of pyrimidine nucleosides can be achieved with reagents such as trifluoromethyl hypofluorite as described by M. J. Robins, Ann New York Acad. Sci. 1975, 255, 104.

d) Modified purine nucleosides may be prepared from the corresponding purine nucleoside derivatives wherein the 2, 6 or 8 substituent is a suitable leaving group such as halogen or sulphonate or 1,3,4-triazole. 6 substituted purine nucleosides may be prepared by treatment of the appropriate 6-halopurine or 6-(1,2,4-triazol-4-yl)-purine nucleoside derivatives with the appropriate nucleophilic reagent as described by V. Nair and A. J. Fassbender Tetrahedron, 1993, 49, 2169 and by V. Samano, R. W. Miles and M. J. Robins, J. Am. Chem. Soc., 1994, 116, 9331. Similarly 8-substituted purine nucleosides can be prepared by treatment of the corresponding 8-halopurine nucleoside with the appropriate nucleophilic reagent as described by L. Tai-Shun, C. Jia-Chong, I. Kimiko and A. C. Sartorelli, J. Med. Chem., 1985, 28, 1481; Nandanan *et al*, J. Med. Chem., 1999,42,1625; J. Jansons, Y. Maurinsh, and M. Lidaks, Nucleosides Nucleotides, 1995, 14, 1709. Introduction of an 8-cyano substituent can be accomplished by displacement using a metal cyanide as described by L-L. Gundersen, Acta. Chem. Scand. 1996, 50, 58. 2-Modified purine nucleoside may be prepared in a similar fashion as described by T. Steinbrecher, C. Wamelung, F. Oesch and A. Seidl, Angew. Chem. Int. Ed. Engl., 1993, 32, 404.

e) Where the substituent at the 2 or 8-position of the purine nucleoside is linked via a carbon carbon bond e. g. alkyl, then metal catalysed cross-coupling procedures can be used starting with the appropriate 2 or 8-halosubstituted purine nucleoside analogue as described by A. A. Van Aerschott, *et al*, J. Med. Chem., 1993, 36, 2938;

V.Nair and G.S. Buenger, J.Am.Chem.Soc., 1989, 111(22), 8502; C. Tu, C. Keane and B. E. Eaton Nucleosides Nucleotides, 1995, 14, 1631.

B. Modification of the carbohydrate moiety:

Following introduction of protecting groups which are compatible with the further chemistry:

- Azide may be introduced at the 4'-position by treatment of the 4',5'-didehydro nucleoside with iodine azide as exemplified by H.Maag *et al*, J. Med.Chem., 1992, 35, 1440. An alkoxide may be introduced at the 4'-position by treatment of the 4',5'-didehydro nucleoside with iodine followed by an alcohol and lead carbonate as exemplified by J.P.Verheyden and J.G.Moffatt, J.Am.Chem.Soc., 1975, 97(15), 4386. Fluoride may be introduced at the 4'-position by treatment of the 4',5'-didehydro nucleoside with iodine followed by silver(I)fluoride as described by G.R.Owen et al, J.Org.Chem., 1976, 41(8), 3010 or A. Maguire *et al*, J. Chem. Soc. Perkin Trans. 1, 1993, 1(15), 1795. A 4'-formyl group can be introduced and subsequently converted to a wide range of substituents including but not limited to 4'-haloalkyl, 4'-ethynyl, 4'-oximinomethyl, and 4'-cyano as exemplified by M. Nomura *et al.*, J. Med. Chem., 1999, 42, 2901.

- Modification of either the 2'-hydroxy substituent or 3'-hydroxy substituent in the nucleoside analogue is possible.

- Conversion of the 3- hydroxy to a leaving group such as halo by reaction with for example triphenyl phosphine and a tetrahaloalkane as described for example by L. De Napoli *et al*, Nucleosides Nucleotides, 1993, 12, 981, followed by reduction provides the 3-deoxysugar derivatives as described by D. G. Norman and C. B. Reese, Synthesis 1983, 304.

- Derivatisation of the 3 hydroxy group by conversion to a triflate group followed by reduction using sodium borohydride as described by S. A. Surzhykov *et al*, Nucleosides Nucleotides, 1994, 13(10), 2283. Direct introduction of a fluorine substituent can be accomplished with fluorinating agents such as diethylamino-sulphur trifluoride as described by P. Herdewijn, A. Van Aerschot and L. Kerremans, NucleosidesNucleotides, 1989,8, 65.

- Conversion of the hydroxy substituent to a leaving group such as halo or sulphonate also allows displacement using nucleophilic reagents such as tetrabutylammonium fluoride, lithium azide, or metal cyanides as exemplified by H. Hrebabecky, A. Holy and E. de Clercq, Collect. Czech. Chem. Comm. 1990, 55,

1800; K. E. B. Parkes and K. Taylor, Tet. Lett., 1988, 29, 2995; H. M. Pfundheller *et al*, Helv. Chim. Acta, 2000, 83, 128.

- Reaction of 2'-keto nucleosides with fluorinating agents such as diethylamino sulfur trifluoride can be used to prepare 2',2'-difluoronucleosides as described by D. Bergstrom, E. Romo and P. Shum Nucleosides Nucleotides, 1987, 6, 53.

2. Construction of the heterocyclic base after glycosylation.

a) those which for example utilise furanosylamine derivatives as described by N. J. Cusack, B. J. Hildick, D. H. Robinson, P. W. Rugg and G. Shaw J. Chem. Soc. Perkin Trans., I 1973, 1720 or G. Shaw, R. N. Warrener, M. H. Maguire and R. K. Ralph, J. Chem. Soc., 1958, 2294.

b) those which utilise for example furanosylureas for pyrimidine nucleoside synthesis as described by J. Šmejkal, J. Farkas, and F. Šorm, Coll. Czech. Chem. Comm., 1966, 31, 291.

c) the preparation of purine nucleosides from imidazole nucleosides is reviewed by L. B. Townsend, Chem. Rev., 1967, 67, 533.

d) the preparation of compounds of formula I wherein X is CH_2 can be accomplished from 1-hydroxymethyl-4-aminocyclopentane derivatives as described by Y. F. Shealy and J. D. Clayton J. Am. Chem. Soc., 1969, 91, 3075; R. Vince and S. Daluge J. Org. Chem., 1980, 45, 531; R. C. Cermak and R. Vince, Tet. Lett., 1981, 2331; R. D. Elliott *et al*, J. Med. Chem., 1994,37, 739.

3. Condensation of a protected furanose, thiofuranose or cyclopentane derivative with a purine or pyrimidine derivative.

The condensation reaction of a protected furanose, thiofuranose or cyclopentane derivative with an appropriate purine or pyrimidine derivative may be performed using standard methods including the use of a Lewis acid catalyst such as mercuric bromide or stannic chloride or trimethylsilyltrifluoromethane sulphonate in solvents such as acetonitrile, 1,2-dichloroethane, dichloromethane, chloroform or toluene at reduced, ambient or elevated temperature. Examples for the condensation reaction of a protected furanose or thiofuranose

- with heavy metal derivatives of purines or pyrimidines derivatives (e. g. chloromercuri derivatives) are described by J Davoll and B. A. Lowry, J. Am. Chem. Soc., 1951, 73, 1650; J. J. Fox, N. Yung, J. Davoll and G. B. Brown, J. Am. Chem. Soc., 1956, 78, 2117.

- with alkoxy pyrimidines are described by K. A. Watanabe, D. H. Hollenberg and J. J. Fox., Carbohydrates. Nucleosides and Nucleotides. 1974, 1,1.

- with silyl derivatives of purines or pyrimidines as described by U. Niedballa and H. Vorbruggen, J. Org. Chem., 1976, 41, 2084; U. Niedballa and H. Vorbruggen, J. Org. Chem., 1974, 39, 3672. A. J. Hubbard, A. S. Jones and R. T. Walker, Nucleic Acids Res., 1984, 12, 6827.

Furthermore

- the fusion of per-acylated sugars with purines under vacuum in the presence of p-toluene sulphonic acid has been described by T. Simadate, Y. Ishudo and T. Sato, Chem. Abs., 1962, 56, 11 692 and W. Pfleiderer, R. K. Robins, Chem. Ber. 1965, 98, 1511.

- the condensation reactions have been described by K. A. Watanabe, D. H. Hollenberg and J. J. Fox, Carbohydrates Nucleosides and Nucleotides, 1974, 1,1.

Examples for the condensation reaction of a protected cyclopentane derivative with an appropriate purine derivative or pyrimidine derivative are given in H. Kapeller, H. Baumgartner and H. Griengl, Monattsh Chem., 1997, 128, 191 and P. Wang *et al*, Tet. Lett., 1997, 38, 4207; or by T. Jenny *et al.* Helv. Chim. Acta, 1992, 25, 1944.

Such methods often result in mixtures of anomeric nucleoside derivatives which can be separated by standard techniques known to the art such as recrystallisation, column chromatography, high performance liquid chromatography or super critical fluid chromatography.

The purine derivatives and pyrimidines derivatives for above condensation reactions can be obtained commercially or can be prepared by procedures known to the art.

The preparation of purine derivatives is reviewed by G. Shaw in "Comprehensive Heterocyclic Chemistry" pub Pergamon Press Vol. 5 chapter 4. 09, p 499 and "Comprehensive Heterocyclic Chemistry II" publ. Pergamon Press, Vol 7, chapter 7. 11, p 397.

The preparation of pyrimidines derivatives is reviewed by D. J. Brown in "The Chemistry of Heterocyclic Compounds – The Pyrimidines" 1962 and Supplement 1, 1970, pub John Wiley and Sons, New York, by D. J. Brown in "Comprehensive Heterocyclic Chemistry" pub Pergamon Press Vol. 5 chapter 4. 09, p 499 and by K.

Unheim and T. Benneche in "Comprehensive Heterocyclic Chemistry II" pub Pergamon Press Vol. 6 chapter 6. 02 p 93.

Furanose derivatives can be prepared from commercially available carbohydrate starting materials such as the D forms of ribose, arabinose, xylose or lyxose, following introduction of protecting groups which are compatible with the chemistry.

4-Substituted furanoses with the substituent containing a carbon attached to the 4-position of the furanose, for example alkyl, alkenyl, alkynyl, haloalkyl, acyl, alkoxycarbonyl, hydroxyalkyl, alkoxyalkyl, cyano, oximinomethyl, alkoxyimino-methyl, alkylaminocarbonyl and acyl can be prepared from the corresponding 4-formyl furanose. The preparation of one such 4-formylfuranose is described by H. Ohrui *et al.*, J. Med. Chem., 2000, 43, 5416. 4-Haloalkyl furanoses may be prepared from the corresponding 4-hydroxymethyl furanoses (e. g., K. Kitano *et al*, Tetrahedron, 1997, 53(39), 13315). 4-Methyl furanoses can be prepared by the method described by T. Waga *et al*, Biosci. Biotech. Biochem. 1993, 19(7), 408.

2,2-Difluorofuranose derivatives can be prepared from D-glucose or D-mannose as described by R. Fernandez, M. I. Mateu, R. Echarri and S. Castillon Tetrahedron, 1998, 54, 3523. The thiofuranose derivatives can be prepared by literature procedures such as L. Bellon, J. L. Barascut, J. L. Imbach, Nucleosides and Nucleotides 1992, 11, 1467 and modified in a similar fashion to the furanose analogues described above.

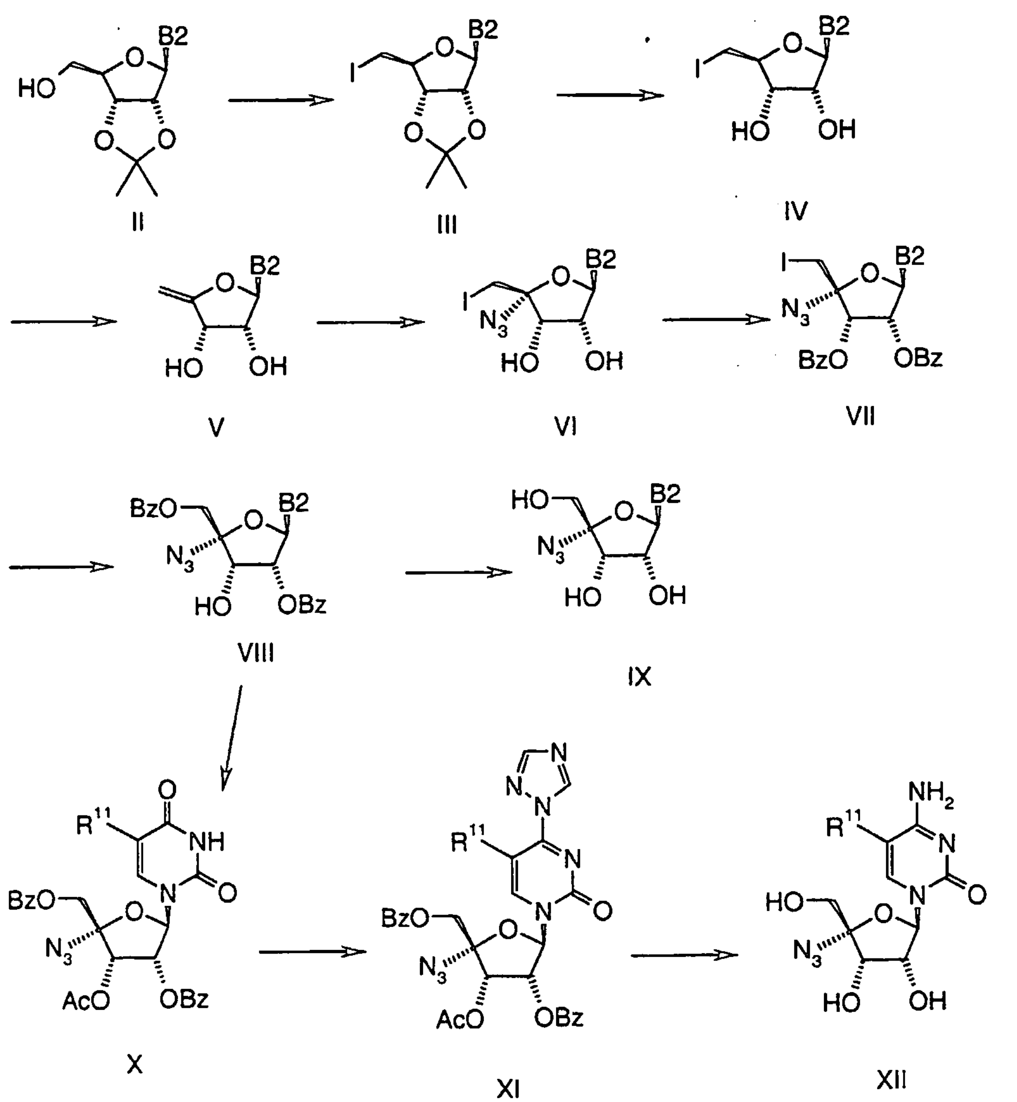
The cyclopentane derivatives can be prepared by methods known in the art of organic chemistry and by methods and references included in L. Agrofolio *et al*, Tetrahedron, 1994, 50, 10611.

The preformed nucleoside derivatives are either available commercially or synthesised in accordance with the methods described above.

The methods discussed above are described in more details below:

The compounds of formula I, wherein R^1 is N_3, R^2 and R^3 are hydroxy and B is B2 can be prepared according to Reaction Scheme A:

Scheme A



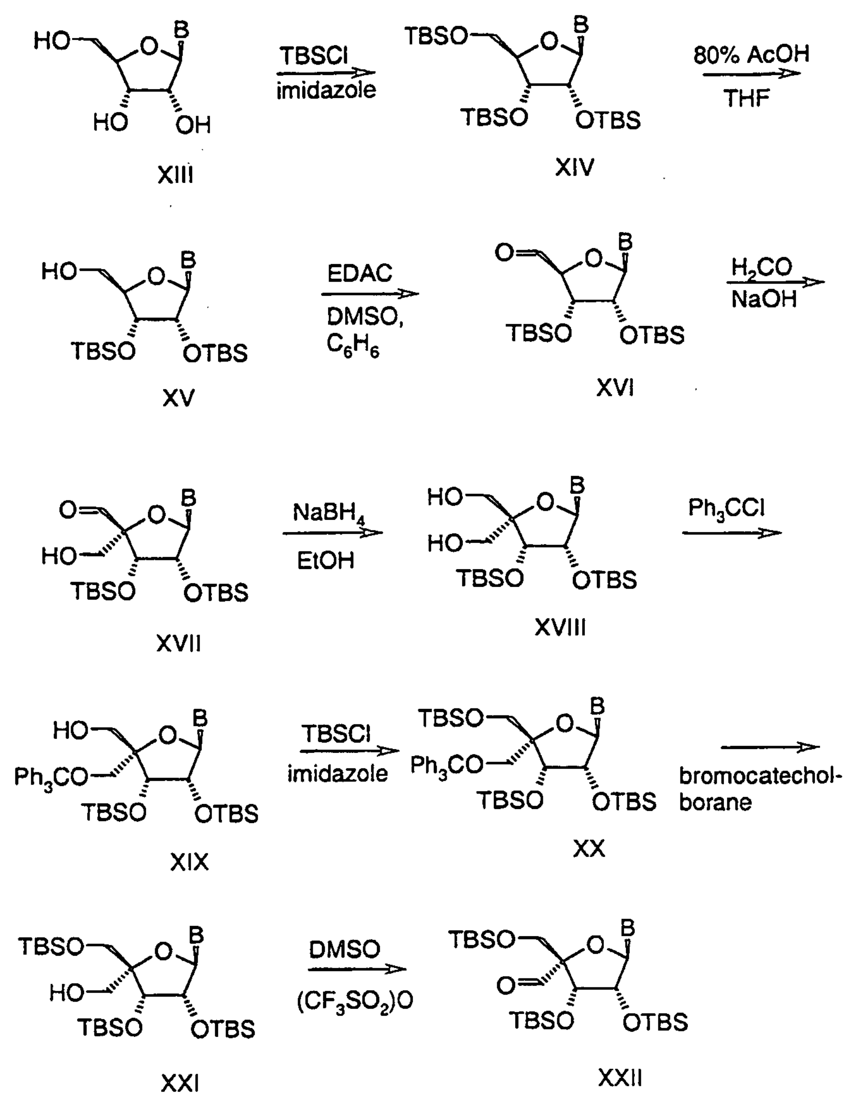
wherein Ac is acetyl, Bz is benzoyl and R^{11} is as defined above.

Compounds of Formula II may be iodinated using a mixture of triphenylphosphine, iodine and pyridine as exemplified by H. Maag *et al*, J. Med. Chem., 1992, 35, 1440. The acetonide protecting group can be removed by treatment with an acid, for instance acetic acid, as described by J. P. Verheyden *et al*, J. Org. Chem., 1970, 35(7), 2319, to give nucleosides of formula III. Following protection of the 2' and 3' hydroxyls with acetic anhydride and pyridine elimination of hydrogen iodide, with for example silver fluoride in pyridine and removal of the acetyl

protecting groups with methanolic ammonia as described by J. P. Verheyden *et al.*, J. Org. Chem., 1974, 39(24), 3573, gives 4',5' didehydro nucleosides of formula V. Addition of iodine azide to the double bond can be accomplished by treatment of V with a mixture of iodine chloride and sodium azide in N,N-dimethylformamide as described by H. Maag *et al*, J. Med. Chem., 1992, 35, 1440, to give nucleosides of formula VI. Protection of the hydroxy groups in VI can be accomplished by treatment of VI with benzoyl chloride in pyridine, giving nucleosides of formula VII, which can then be converted into the 5'-benzoyl nucleosides of formula VIII by treatment with *meta*-chloroperbenzoic acid in dichloromethane, which can then be deprotected with a base, eg sodium methoxide, in methanol to give nucleosides of formula IX, all as described by H. *Maag et al*, J. Med. Chem., 1992, 35, 1440. In the case where B2 in the compound of formula VIII is uracil or 5'-substituted uracil, following protection of the 3'-hydroxy group with acetic anhydride and pyridine, conversion to the corresponding cytidine of formula XII can be accomplished by the method described by A. D. Borthwick *et al.*, J. Med. Chem., 1990, 33(1), 179, whereby nucleosides of formula X can be treated with 4-chlorophenyl dichlorophosphate and triazole to give 4-triazolyl nucleosides of formula XI, followed by treatment of nucleosides XI with aqueous ammonia giving 5-substituted cytidines of formula XII.

Compounds of formula I, wherein R^1 is -C≡CH, -CH=CHCl, -CH=N-OH, -CN, R^2 and R^3 are hydroxy and B is B1 or B2 can be prepared according to Reaction Scheme B.

Scheme B

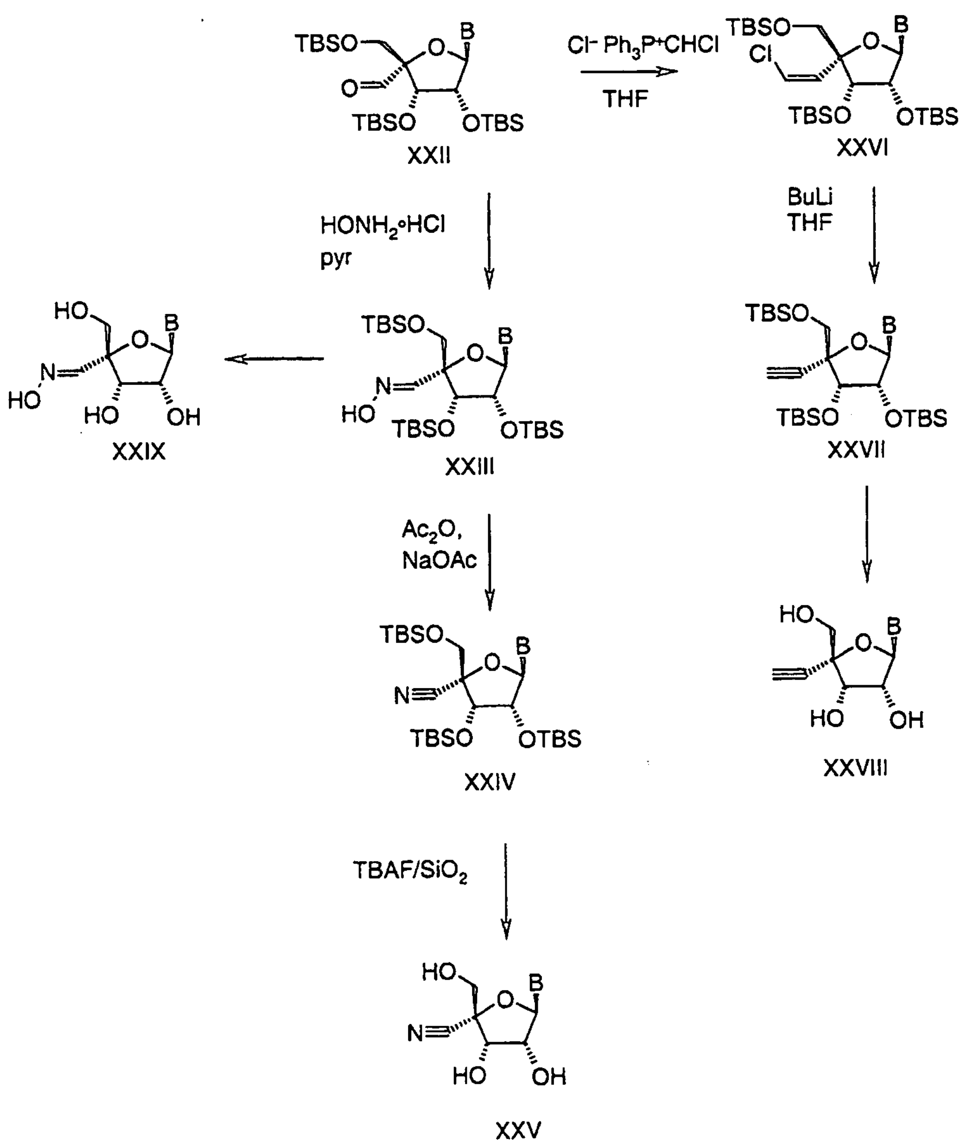


Compounds of formula XIII can be silylated with *tert*-butyldimethylsilylchloride (TBSCl) and imidazole to give the tri-*tert*-butyldimethylsilyl compounds of formula XIV. The 5'-*tert*-butyldimethylsilyl ether can be deprotected using 80% acetic acid to give the 5-hydroxy nucleosides XV, which can then be oxidised to the 5'-formyl nucleosides XVI using a mixture of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC) and dimethylsulphoxide (DMSO) in a suitable solvent, eg benzene. Alkylation of XVI with formaldehyde and sodium hydroxide gives the 4'-hydroxymethyl compounds XVII which can be reduced to the 4'-

dihydroxymethyl compounds XVIII. Selective protection of the hydroxymethyl on the α face of the nucleoside with trityl chloride in pyridine gives the 4'-trityl compounds XIX, followed by protection of the hydroxymethyl on the β-face of the nucleoside with *tert*-butyldimethylsilylchloride (TBSCl) and imidazole gives compounds of formula XX. Deprotection of the trityl group with bromocatechol-borane gives the 4'-hydroxymethyl compound XXI, which can be oxidised with trifluoromethanesulphonic anhydride and dimethylsulphoxide to give the 4'-formyl compound of formula XXII.

The aldehyde of formula XXII can be used as starting material for a wide range of 4'-substituted nucleosides as depicted in Scheme C:

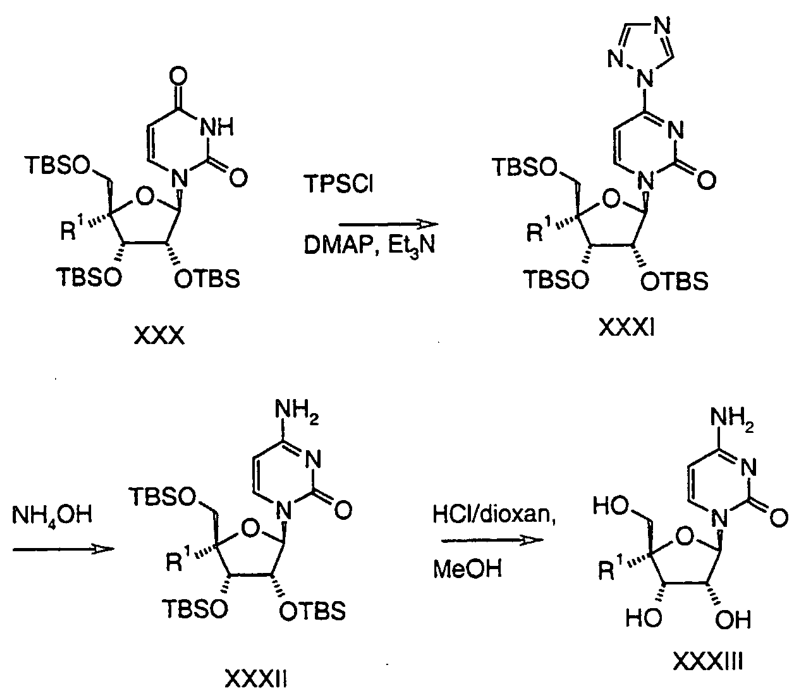
Scheme C



Treatment of the aldehyde XXII with hydroxylamine hydrochloride and pyridine gives the 4'-hydroxyimine of formula XXIII. Water is eliminated from compound XXIII to give 4'-cyano compounds of formula XXIV. Treatment of 4'-formyl compounds of formula XXII with chloromethylphosphonium chloride and butyl lithium gives the 4'-(2-chloroethenyl) compounds XXVI. Treatment of compounds XXVI with butyllithium results in the elimination of hydrogen chloride to give the 4'-ethynyl compounds of formula XXVII. Removal of the silyl protecting groups from the tri *tert*-butyldimethylsilylchloride protected compounds XXIII, XXVII and XXIV can be carried out with a fluoride source such as ammonium flouride in methanol or tetrabutylammonium fluoride absorbed on silica in tetrahydrofuran, to give the respective 4'-substituted nucleosides XXV, XXVIII and XXIX.

Suitably protected 4'-substituted uridines (for example XXIV and XXVII) can be converted to the corresponding 4'-substituted cytidines according to Reaction Scheme D.

Scheme D



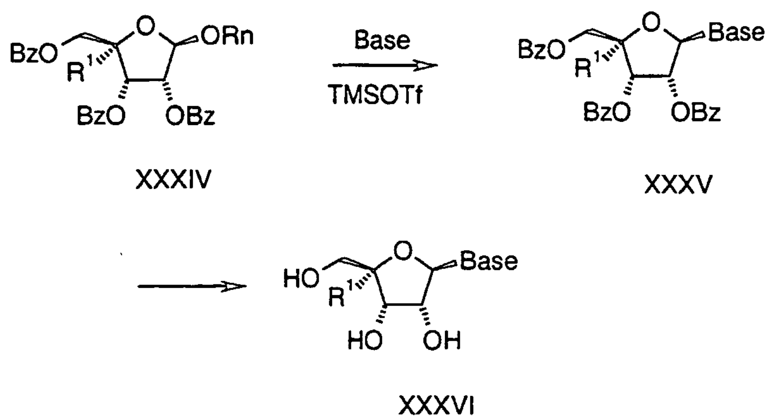
The tri-*tert*butyldimethylsilyl (TBS) protected uridines of formula XXX can be treated with tri-isopropylbenzenesulphonyl chloride, triethylamine and dimethylaminopyridine to give the 4-triazolylnucleosides XXXI. The 4-triazolyl compounds XXXI can be converted to the 4-amino compounds XXXII with

aqueous ammonia. Deprotection of the silyl groups with a mixture of methanol and hydrochloric acid in dioxan gives the cytidine derivatives XXXIII.

Compounds of formula I, wherein R^1 is alkoxy, R^2 and R^3 are hydroxy and B is a 9-purinyl residue B1 or a 1-pyrimidyl residue B2 can be prepared according to the procedures described by J.P. Verheyden *et al.* US patent no. 3 910 885

Compounds of formula I in which R^1 is trifluoromethyl, methyl or ethynyl can be prepared as depicted in Reaction Scheme E:

Scheme E



for example by coupling the appropriate protected 4'-substituted ribofuranoside XXXIV with a silylated base in the presence of a Lewis acid, eg trimethylsilyltrifluoromethanesulphonate (TMSOTf) or tin tetrachloride, in an appropriate solvent, eg acetonitrile or 1,2-dichloroethane, to give compound of formula XXXV. The protecting groups can be removed by treatment of XXXV with a base, for example sodium methoxide, in compatible solvent for instance methanol to give compounds of formula XXXVI.

Methods for the monophosphorylation of organic compounds including nucleosides have been reviewed by L A Slotin, Synthesis, 1977, 737. More recently other nucleoside phosphorylation procedures have been described: M Uchiyama et al J. Org. Chem., 1993, 58,373; R Caputo et al, Synlett., 1997, 739 and M Taktakishvili and V Nair Tet. Lett. 2000, 41, 7173. Other procedures for monophosphorylation that may be useful for nucleosides are described by C E McKenna and J Schmidhauser, J. Chem. Soc., Chem. Commun., 1979, 739 and J K Stowell and T S Widlanski Tet. Lett., 1995, 1825. Synthesis of di and triphosphate

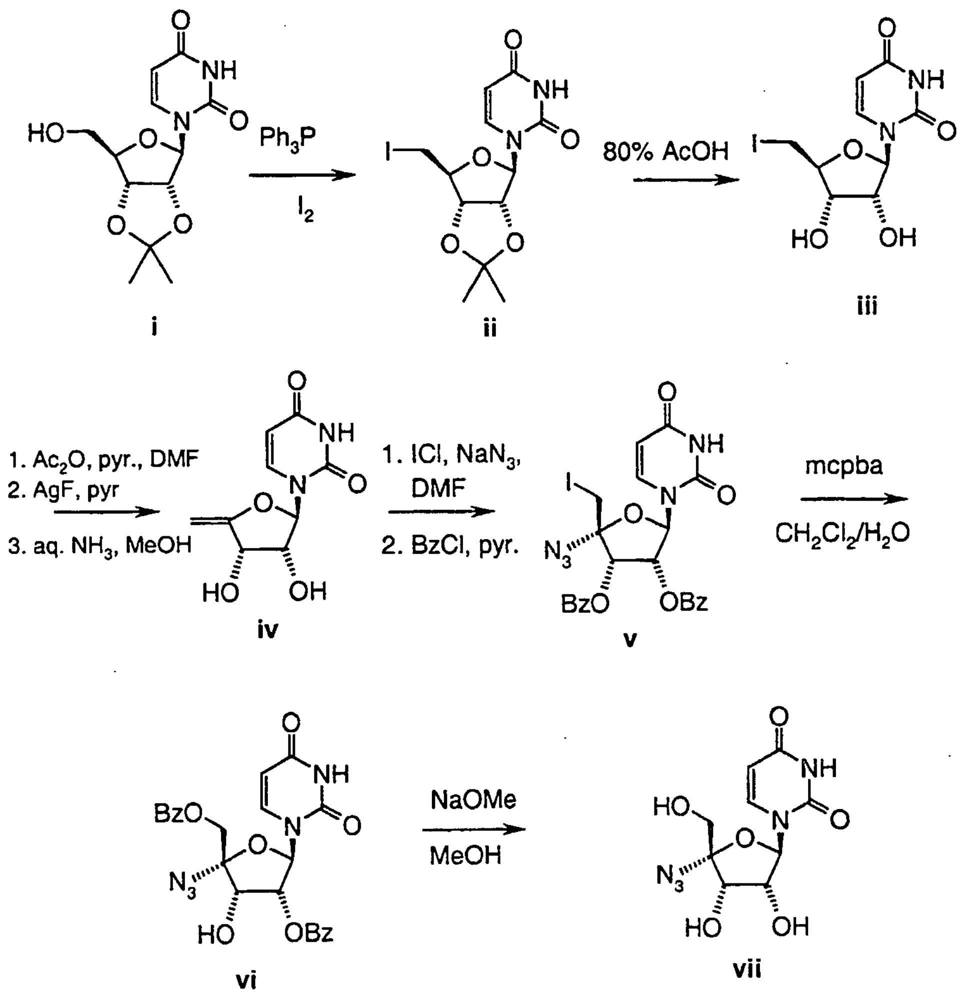
derivatives are reviewed in K H Scheit, Nucleotide Analogues, 1980, Wiley Interscience and by K Burgess and D Cook Chemical Reviews, 2000, 100, 2047.

The following Examples illustrate methods for the preparation of compounds of formula I:

Example 1

Preparation of compound 1, according to the method of schemes 1 and 1a

Scheme 1



1.1. Compound **(i)**

Compound **(i)** was purchased from Lancaster (Cat. no.: 206-647-7, CAS 362-43-6)

1.2. Compound **(ii)**

Triphenylphosphine (1.57 g, 6.0 mmol) and iodine (1.52 g, 6.0 mmol) were added to compound **(i)** (1.14 g, 4.0 mmol) in dioxan (20 ml) containing pyridine

(0.65 mmol, 8.0 mmol). The mixture was stirred overnight and quenched with methanol (1 ml). The solvent was evaporated *in vacuo*. The residue was dissolved in ethyl acetate (200 ml), washed with water (100 ml), 10% aqueous sodium thiosulphate (100 ml), brine (100 ml) and dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel, eluting with 1:1 ethyl acetate/petrol) to afford compound **(ii)** as a colourless oil which slowly solidified to a colourless waxy solid (1.5 g) mass spectrum (CI) m/z 395 $[M+H]^+$.

1.3. Compound **(iii)**

Compound **(iii)** was prepared from compound **(ii)** as described by J. P. Verheyden *et al.*, J. Org. Chem., 1970, 35(7), 2319.

1.4. Compound **(iv)**

Compound **(iv)** was prepared from compound **(iii)** as described by J. P. Verheyden *et al.*, J. Org. Chem., 1974, 39(24), 3573.

1.5. Compound **(v)**

Compound **(v)** was prepared from compound **(iv)** as described by H. Maag *et al.*, J. Med. Chem., 1992, 35, 1440-1451.

1.6. Compound **(vi)**

To a solution of compound **(v)** (482 mg, 0.80 mmol) in dichloromethane saturated with water (10 ml) was added 55% metachloroperbenzoic acid (1.0g, 4.95 mmol). The mixture was stirred for 2 h. Additional metachloroperbenzoic acid (0.50 g) was added and the mixture was stirred for an additional 3 h. Ethyl acetate (100 ml) was added and the solution washed with 10% sodium metabisulphite solution (50 ml), followed by saturated sodium hydrogen carbonate solution (50 ml). The ethyl acetate was dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was subjected to flash chromatography, eluting with 1:1 ethyl acetate/ petrol 1:1 to afford compound **(vi)** as a colourless glass (200 mg); mass spectrum (ESI) m/z 535 $[M+H+CH_3CN]^+$

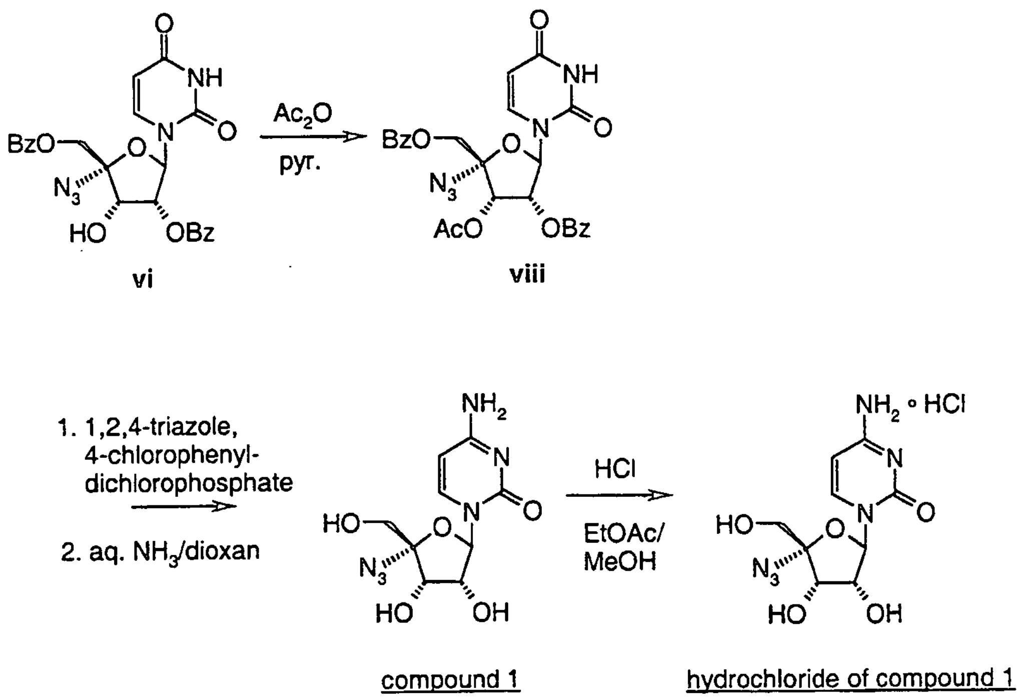
1.7. Compound **(vii)**

To a solution of compound **(vi)** (170 mg, 0.35 mmol) in methanol (2 ml) was added a solution of sodium methoxide in methanol (0.5 M, 0.5 ml). The solution

was stirred for 2 h at room temperature. The solution was neutralised with ion exchange resin (Amberlite IRC 50 (H^+), Aldrich, cat. no. 42,883-3) and stirred for 10 min. The resin was removed by filtration. The filtrate was evaporated *in vacuo* and the residue was subjected to flash chromatography eluting with 1:1 ethyl acetate/ acetone) to afford a colourless oil. Trituration with ethyl acetate afforded compound **(vii)** as a colourless solid (35 mg); mass spectrum (CI) m/z 286 $[M+H]^+$.

The tranformation of the azidouridine derivative to the corresponding azidocytidine derivative (compound 1) and its hydrochloride salt is depicted in Scheme 1a

Scheme 1a



vi

viii

compound 1

hydrochloride of compound 1

1.8. Compound **(viii)**

To a solution of compound **(vi)** (460 mg, 0.93 mmol) in pyridine (3 ml) was added acetic anhydride (1 ml) and the mixture was stirred for 4 h. Ethyl acetate (100 ml) was added and the mixture was washed with 2 N HCl (50 ml), followed by saturated sodium hydrogen carbonate solution (50 ml). The solution was dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was subjected to

flash chromatography eluting with 1:1 ethyl acetate/ petrol to afford compound **(viii)** as a colourless gum (350 mg); mass spectrum (ESI) m/z 536 $[M+H]^+$

1.9. Compound 1

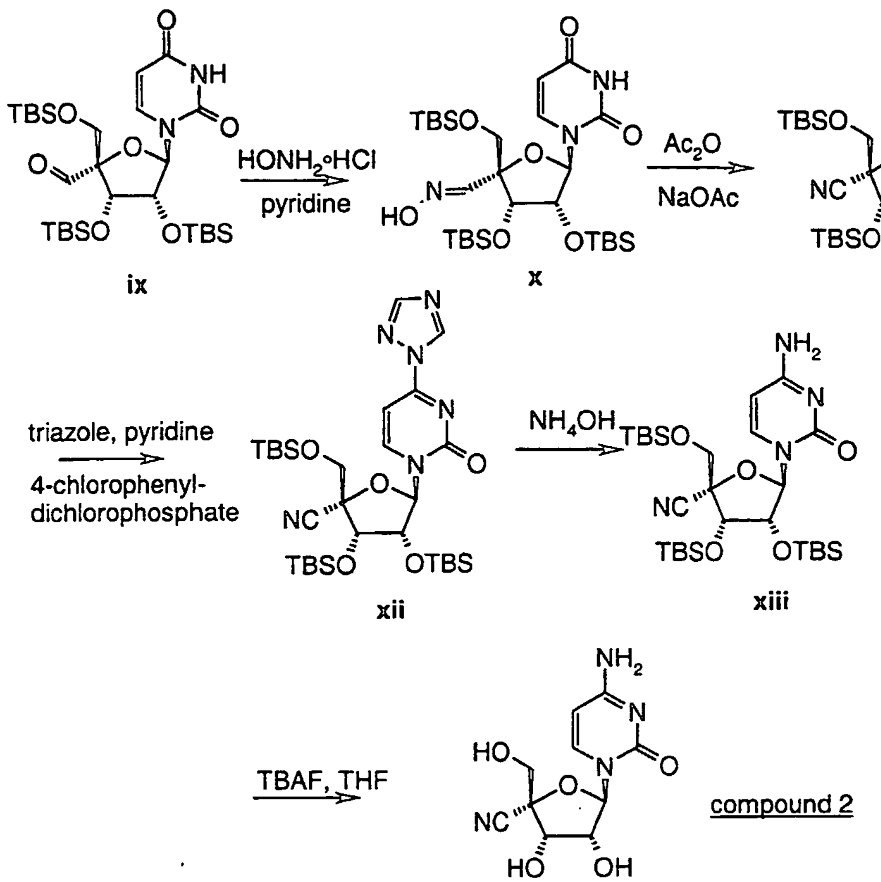
To a solution of compound **(viii)** (1.5 g, 2.8 mmol) in pyridine (20 ml) was added 1,2,4-triazole (0.97 g, 14 mmol). 4-chlorophenyldichlorophosphate (1.36 ml, 8.4 mmol) was then added dropwise with stirring. The mixture was stirred for 16 h. Ethyl acetate (300 ml) was added and the mixture was washed with saturated sodium hydrogen carbonate solution (200 ml). The solution was dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was subjected to flash chromatography eluting with 2:1 ethyl acetate/ petrol to afford a yellow foam (850 mg). The foam was treated with dioxan (8 ml) followed by aqueous ammonia solution (16 ml) and stirred for 16 h. The filtrate was evaporated *in vacuo* and the residue was subjected to flash chromatography eluting with 90:18:3:2 dichloro-methane/methanol/acetic acid/water to afford compound 1 as a light tan foam (350 mg); mass spectrum (FAB) m/z 285 $[M+H]^+$

1.10. Hydrochloride of Compound 1

Compound 1 (0.40g) was dissolved in methanol and treated with a solution of hydrogen chloride in ethyl acetate. The product separated as a microcrystalline solid and was collected by filtration and dried *in vacuo* to afford the hydrochloride salt of compound 1 (0.22g); mass spectrum (ESI) m/z 285 $[M+H]^+$

Example 2

Preparation of compound 2 according to the method of scheme 2



2.1. Compound (ix)

Compound (ix) was prepared from compound (xiv), see example 3, as described by M. Nomura *et al.*, J. Med. Chem., 1999, 42, 2901-2908.

2.2. Compound (x)

A mixture of (ix) (600 mg, 0.98 mmol) and hydroxylamine hydrochloride (140 mg, 1.95 mmol) in pyridine was stirred at room temperature for 2 h. The reaction mixture was evaporated *in vacuo* and the residue was partitioned between ethyl acetate (30 ml) and water (30 ml). The ethyl acetate layer was separated and dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate evaporated *in vacuo* to afford compound (x) as a white foam (615 mg); mass spectrum (ESI) m/z 630 $\{M+H]^+$.

2.3. Compound **(xi)**

A mixture of compound **(x)** (550 mg, 0.87 mmol) and sodium acetate (720 mg, 5.25mmol) was suspended in acetic anhydride then heated at 130°C for 3 h. The reaction mixture was evaporated *in vacuo* and the residue partitioned between ethyl acetate (30 ml) and saturated sodium bicarbonate (30 ml). The ethyl acetate layer was separated and dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel, eluting with 1:2 diethyl ether/hexane. Product containing fractions were combined and evaporated *in vacuo* to afford compound **(xi)** as a colourless solid (285 mg). mass spectrum (ESI) m/z 612 $[M+H]^+$.

2.4. Compound **(xii)**

4-chlorophenyl-dichlorophosphate (160 µL, 0.98 mmol) was added dropwise to a solution of compound **(xi)** (200 mg, 0.33 mmol) and 1,2,4-triazole (115 mg, 1.63 mmol) in anhydrous pyridine (5 ml) then stirred at room temperature for 16 h. The reaction mixture was evaporated *in vacuo* and the residue partitioned between ethyl acetate (30 ml) and 2M hydrochloric acid (30 ml). The ethyl acetate layer was separated, washed with saturated sodium bicarbonate (30 ml) and dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel, eluting with 1:1 diethyl ether/hexane followed by 2:1 diethyl ether/hexane. Product containing fractions were combined and evaporated *in vacuo* to afford **(xii)** as a cream solid (65 mg). mass spectrum (ESI) m/z 663 $[M+H]^+$.

2.5. Compound **(xiii)**

A solution of compound **(xii)** (60 mg, 0.09mmol) and aqueous ammonia (2 ml) in acetonitrile was stirred at room temperature for 16 h. The reaction mixture was evaporated *in vacuo* and the residue partitioned between ethyl acetate (10 ml) and 2 M hydrochloric acid (10 ml). The ethyl acetate layer was separated and dried over magnesium sulphate. The magnesium sulphate was removed by filtration and evaporated *in vacuo* to afford compound **(xiii)** as a pale yellow solid (45 mg); mass spectrum (ESI) m/z 611 $[M+H]^+$

2.6. Compound 2

Tetrabutylammonium fluoride (1 M solution in THF, 0.3 ml) was added to a stirred solution of compound **(xiii)** (40 mg, 0. 06 mmol) in dry tetrahydrofuran (10 ml) and stirred at room temperature for 2h. The solvent was removed by evaporation *in vacuo* The residue was treated with pyridine (1ml) followed by acetic anhydride (0.3ml) and stirred for 4h at room temperature. The solvent was removed by evaporation *in vacuo.* The residue was treated with ethyl acetate (50ml) and washed with dilute hydrochloric acid (30ml) followed by a 5% aqueous sodium bicarbonate solution. The ethyl acetate was dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate evaporated *in vacuo.* The residue was subjected to flash column chromatography eluting with ethyl acetate to afford an oil. The oil was dissolved in methanol (1ml) and treated with sodium methoxide (0.5M solution in methanol, 0.05ml) and stood at room temperature for 3h. The mixture was neutralised with ion exchange resin (Amberlite IRC 50 (H^+). The resin was removed by filtration, and the filtrate evaporated *in vacuo*. The residue was disolved in water and freeze dried to afford compound 2 as an amorphous solid (7mg).

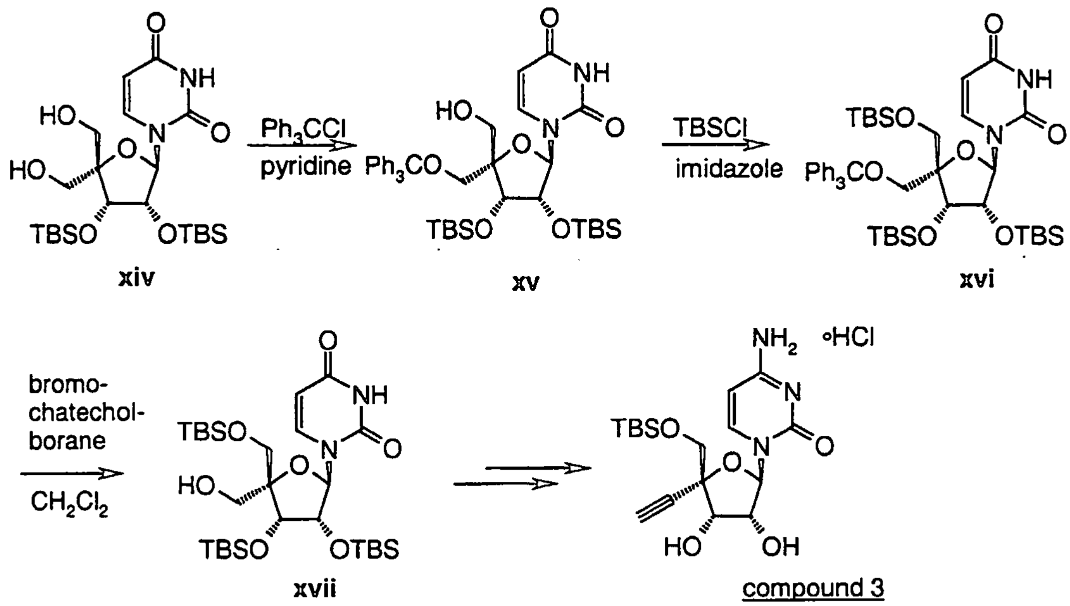
2.7. The corresponding 4'-cyanouridine can be prepared by deprotection of compound **(xi)**.

The deprotection can be carried out as follows:

Compound **(xi)** (50 mg, 82 μmol) was dissolved in tetrahydrofuran, treated with tetrabutylammonium fluoride on silica then stirred for 16 h at room temperature. The reaction mixture was filtered through Hyflo Super Cel (Fluka, cat no. 56678), evaporated *in vacuo*, then purified by flash column chromatography on silica gel, eluting with dichloromethane/methanol/acetic acid/water (240:24:3:2) followed by dichloromethane/methanol/acetic acid/water (90:18:3:2). Product containing fractions were combined and evaporated. The residue was dissolved in methanol/water (5:1), treated with Duolite C225 ion exchange resin (H^+ form, BDH, cat. no. 56678) and stirred for 15 min. The resin was removed by filtration and the filtrate evaporated *in vacuo* to low volume. The product was collected by filtration and dried *in vacuo* to afford 4'-cyanouridine as a white crystalline solid (15 mg); mass spectrum m/z (ESI) 270 $[M+H]^+$.

Example 3

Preparation of compound 3 according to the method of Scheme 3



3.1. Compound **(xiv)**

This compound was prepared as described by M. Nomura *et al.*, J. Med. Chem., 1999, 42, 2901-2908.

3.2. Compound **(xv)**

Trityl chloride (3.2 g; 11.5 mmol) was added to a solution of compound **(xiv)** (3.0 g, 6.0 mmol) in pyridine (20 ml) and stirred at room temperature for 16 h. The solvent was evaporated *in vacuo* and the residue partitioned between ethyl acetate (50 ml) and 2 M hydrochloric acid (50 ml). The ethyl acetate layer was separated, washed with brine (50 ml) and dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate evaporated *in vacuo*. The crude material was purified by flash column chromatography on silica gel, eluting with 2:1 diethyl ether/hexane. Product containing fractions were combined and evaporated *in vacuo* to afford compound **(xv)** as a white solid (2.75 g); mass spectrum (ESI) m/z 767 $[M+H]^+$.

3.3. Compound **(xvi)**

tert-Butyldimethylsilylchloride (0.67 g, 4.4 mmol) and imidazole (0.91 g, 13.3 mmol) was added to a stirred solution of compound **(xv)** (2.75 g, 3.7 mmol) in dimethylformamide (20 ml). The reaction was heated to 45°C for 16 h.

Additional *tert*-butyldimethylsilylchloride (0.67 g, 4.4 mmol) and imidazole (0.91g, 13.3 mmol) were added and the mixture was heated to 60°C for 4 h. The solvent was removed by evaporation *in vacuo* and the residue was partitioned between ethyl acetate and brine. The ethyl acetate was separated and washed with more brine and dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate evaporated *in vacuo*. The residual colourless foam was purified by flash column chromatography on silica gel, eluting with 1:2 diethyl ether/hexane. Product containing fractions were combined and evaporated *in vacuo* to afford compound **(xvi)** as a white solid (3.1 g).

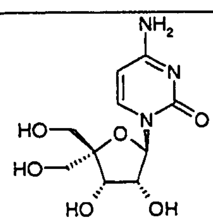
3.4. Compound **(xvii)**

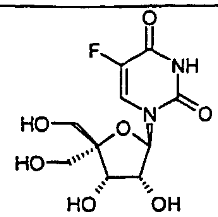
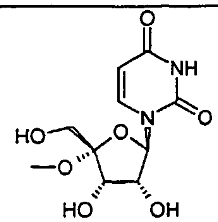
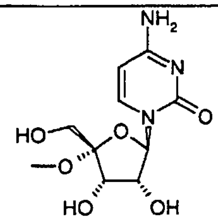
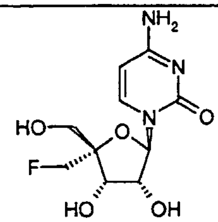
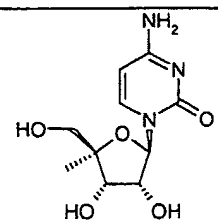
Bromocatecholborane (355 mg, 1.77 mmol) was added to a stirred solution of compound **(xvi)** (1.5 g, 1.77 mmol) in dry dichloromethane (50 ml), under a nitrogen atmosphere at 0°C. The reaction was stirred for 15 min, diluted with dichloromethane (50 ml) then washed with saturated sodium bicarbonate (100 ml) and brine (100 ml). The dichloromethane was dried over anhydrous magnesium sulphate. The magnesium sulphate was removed by filtration and the filtrate evaporated *in vacuo*. The residue was purified by flash column chromatography on silica gel, eluting with 1:1 diethyl ether/hexane. Product containing fractions were combined and evaporated *in vacuo* to afford compound **(xvii)** as a white solid (930 mg).

3.5. Compound 3

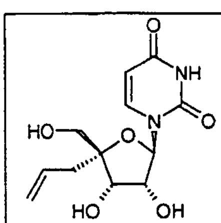
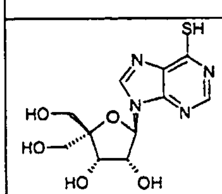
was prepared from compound **(xvii)** as described by M. Nomura *et al.*, J. Med. Chem., 1999, 42, 2901-2908.

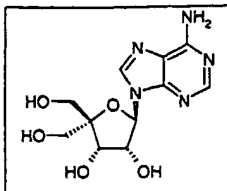
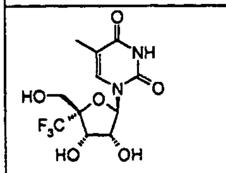
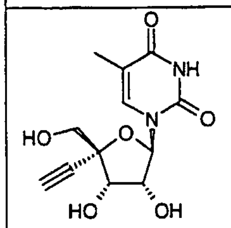
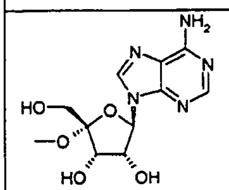
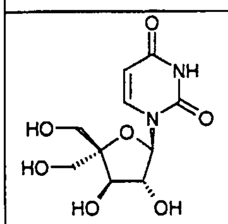
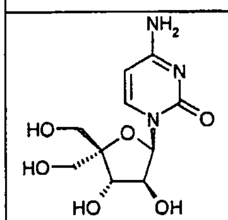
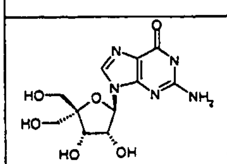
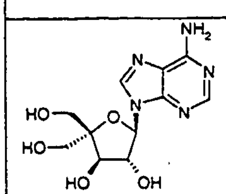
Further compounds can be prepared according to the methods described in the art, for example:

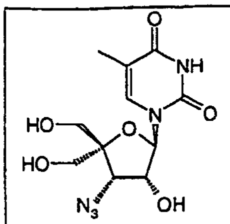
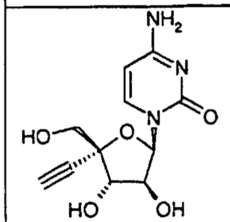
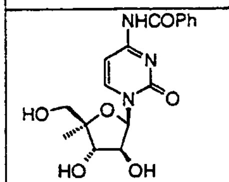
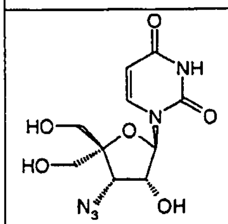
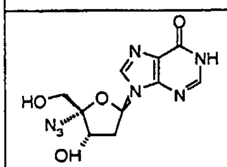
Compound no.	Structure	Name and preparation method
compound 6		4'-C-(Hydroxymethyl)cytidine G. H. Jones *et al.*, J. Org. Chem., 1979, 44(8), 1309.

compound 7		5-Fluoro-4'-C-(hydroxymethyl)uridine Youssefyeh *et al.*, J. Org. Chem., 1979, 44, 1301.
compound 8		4'-C-Methoxyuridine J. A. Cook and J. L. Secrist, J. Am. Chem. Soc., 1979, 101, 1554
compound 9		4'-C-Methoxycytidine J. G. Moffatt and J. P. Verheyden, US patent no. 3 910 885
compound 22		4'-C-(Fluoromethyl)cytidine K. Kitano *et al.*, Tetrahedron, 1997, 53(39), 13315.
compound 23		4'-C-Methylcytidine T. Waga *et al.*, J. Biosci. Biotechnol. Biochem., 1993, 57(9), 1433

Additional compounds of formula I can be prepared in analogy to the methods described in the prior art listed below:

	4'-C-Allyluridine J. Secrist *et al.*, J. Am. Chem. Soc., 1978, 100, 2554.
	9-[4-C-(Hydroxymethyl)-beta-D-ribofuranosyl]-6-mercaptopurine Youssefyeh *et al.*, J. Org. Chem., 1979, 44, 1301

	4'-C-(Hydroxymethyl)adenosine A. Rosenthal and M. Ratcliffe, Carbohydr. Res., 1977, 54, 61.
	4'-C-(Trifluoromethyl)-5-methyluridine J. Kozak and C. R. Johnson 1998, 17(12), 2221.
	4'-C-(Ethynyl)-5-methyluridine R. Yamaguchi *et al.*, J. Biosci. Biotechnol. Biochem.,1999, 63(4), 736
	4'-C-Methoxyadenosine C. M. Richards *et al.*, Carbohydr. Res., 1982, 100, 315.
	1-[4-C-(Hydroxymethyl)-beta-D-xylofuranosyl]uracil G. H. Jones *et al.*, J. Org. Chem., 1979, 44(8), 1309-1317
	1-[4-C-(Hydroxymethyl)-beta-D-arabinofuranosyl]cytosine T. Waga *et al.*, Nucleosides Nucleotides, 1996, 15(1-3) 287-304
	4'-C-(Hydroxymethyl)guanosine J. C. Martin and J. P. Verheyden, Nucleosides Nucleotides 1988, 7(3), 365
	9-[4-C-(Hydroxymethyl)-beta-D-xylofuranosyl]adenine D. L. Leland and M. P. Kotick, Carbohydr. Res., 1974, 38, C9-C11

	3'-Azido-3'-deoxy-4'-C-(hydroxymethyl)-5-methyluridine A. G. Olsen *et al*, J. Chem. Soc. Perkin Trans. 1, 2000, 21, 3610
	1-(4-C-Ethynyl-beta-D-arabinofuranosyl)cytosine H. Ohrui *et al*, J. Med. Chem., 2000, 43(23), 4516 or S. Kohgo *et al.*, Biosci. Biotechnol. Biochem., 1999, 63(6), 1146
	N4-Benzoyl-1-[4-C-methyl-beta-D-arabinofuranosyl]cytosine T. Yamaguchi *et al.*, Nucleosides Nucleotides, 1997, 16(7), 1347
	3'-Azido-3'-deoxy-4'-C-(hydroxymethyl)uridine S. A. Surzhikov and N. B. Dyatkina Russ. J. Biorg. Chem. (Engl. Transl.), 1993, 19(7), 408
	Preparation of 2'-deoxy-4'-azidonucleosides H. Maag, *et al.* Eur. Pat. Appl. EP 371366 A1

The following assay method demonstrates the ability of the compounds of formula I to inhibit HCV RNA replication, and therefore their potential utility for the treatment of HCV infections.

Renilla luciferase assay

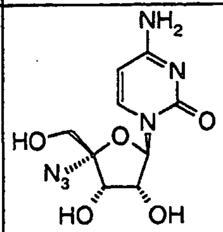
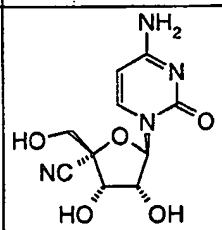
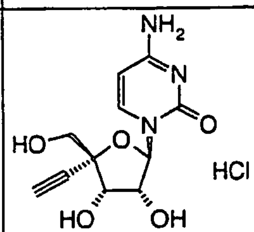
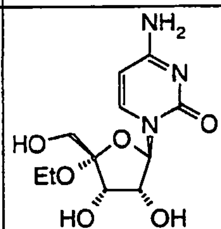
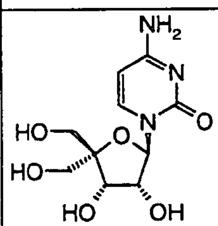
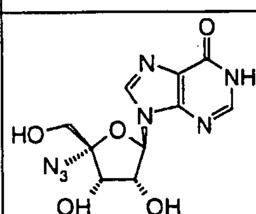
This assay is based on the idea of using a reporter as a simple readout for intracellular HCV replicon RNA level. For this purpose Renilla luciferase gene was introduced into the first open reading frame of a replicon construct NK5.1 (Krieger *et al.*, J. Virol. 75:4614), immediately after the internal ribosome entry site (IRES) sequence, and fused with the neomycin phosphotransferase (NPTII) gene via a self-cleavage peptide 2A from foot and mouth disease virus (Ryan & Drew, EMBO Vol 13:928-933). After *in vitro* transcription the RNA was electroporated into human hepatoma Huh7 cells, and G418-resistant colonies were isolated and expanded.

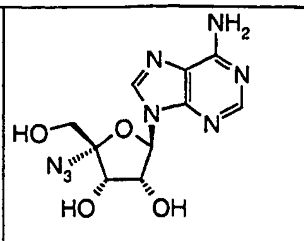
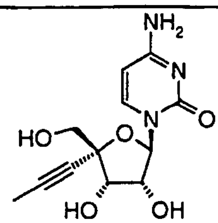
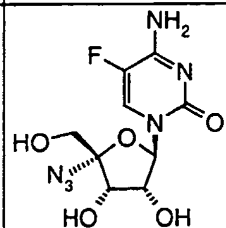
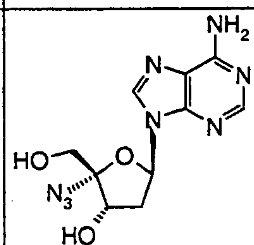
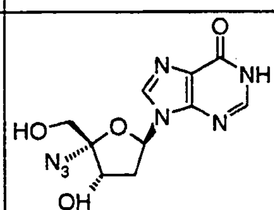
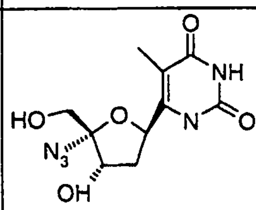
Stably selected cell line 2209-23 was shown to contain replicative HCV subgenomic RNA, and the activity of Renilla luciferase expressed by the replicon reflects its RNA level in the cells.

For the assay procedure, Renilla Luciferase HCV replicon cells (2209-23) that cultured in Dulbecco's MEM (GibcoBRL cat no. 31966-021) with 5% fetal calf serum (FCS, GibcoBRL cat. no. 10106-169) were plated onto a 96-well plate at 5000 cells per well, and incubated overnight. Twenty-four hours later, different dilutions of chemical compounds in the growth medium were added to the cells, which were then further incubated at 37°C for three days. The assay was carried out in duplicate plates, one in opaque white and one in transparent, in order to measure the activity and cytotoxicity of a chemical compound in parallel ensuring the activity seen is not due to reduction on cell proliferation.

At the end of the incubation time, the cells in white plates were harvested and luciferase activity was measured by using Dual-Luciferase reporter assay system (Promega cat no. E1960) All the reagents described in the following paragraph were included in the manufacturers kit, and the manufacturer's instructions were followed for preparations of the reagents. Briefly, the cells were washed twice with 200 μl phosphate buffered saline (pH 7.0) (PBS) per well and lysed with 25 μl of 1x passive lysis buffer prior to incubation at room temperature for 20 min. One hundred microlitre of LAR II reagent was added to each well. The plate was then inserted into the LB 96V microplate luminometer (MicroLumatPlus, Berthold), and 100 μl of Stop & Glo reagent was injected into each well by the machine and the signal measured using a 2-second delay, 10-second measurement program. IC50, the concentration of the drug required for reducing replicon level by 50% in relation to the untreated cell control value, can be calculated from the plot of percentage reduction of the luciferase activity vs. drug concentration. The results are compiled below.

For the cytotoxicity assay, WST-1 reagent from Roche Diagnostic (cat no. 1644807) was used. Ten microlitre of WST-1 reagent was added to each well including wells that contain media alone as blanks. Cells were then incubated for 1 to 1.5 hours at 37°C, and the OD value was measured by a 96-well plate reader at 450nm (reference filter at 650nm). Again CC50, the concentration of the drug required for reducing cell proliferation by 50% in relation to the untreated cell control value, can be calculated from the plot of percentage reduction of the WST-1 value vs. drug concentration.

Compound no.	STRUCTURE	Name	IC50 (μM)	CC50(μM) WST-1
compound 1		4'-C-Azidocytidine	1.2	0% (100 μM)
compound 2		4'-C-Cyanocytidine	99 (20 μM)	100% (20μM)
compound 3		4'-C-Ethynyl-cytidine hydrochloride (1:1)	3% (20 μM)	0% (20 μM)
compound 4		4'-C-Ethoxy-cytidine	11% (20μM)	0% (20μM)
compound 6		4'-C-(Hydroxy-methyl)-cytidine	13% (20μM)	2% (20μM)
compound 16-1		4'-C-Azidoinosine	>500μM	

compound 18		4'-C-Azido-adenosine	57	
compound 30		4'-C-(1-Propynyl)-cytidine	15% (20μM)	2% (20μM)
compound 44		4'-C-Azido-5-fluorocytidine	108	
compound 46		4'-Azido-2'-deoxyadenosine	13	0% (20μM)
compound 47		4'-C-Azido-2'-deoxy inosine	37	12% (20μM)
compound 48		4'-C-Azido- 5-methyluridine	8	0% (20μM)

As shown in above Table the compounds of formula I have the potential to be efficacious as antiviral drugs for the treatment of HCV infections in humans, or are metabolized to a compound that exhibit such activity.

In another embodiment of the invention, the active compound or its derivative or salt can be administered in combination with another antiviral agent,

such as an anti-hepatitis agent, including those of formula I. When the active compound or its derivative or salt are administered in combination with another antiviral agent the activity may be increased over the parent compound. This can easily be assessed by preparing the derivative and testing its anti-HCV activity according to the method described herein.

Administration of the active compound may range from continuous (intravenous drip) to several oral administrations per day (for example, Q.I.D) and may include oral, topical parenteral, intramuscular, intravenous, subcutaneous, transdermal (which may include a penetration enhancement agent), buccal and suppository administration, among other routes of administration.

The 4'-substituted nucleoside derivatives as well as their pharmaceutically useable salts, can be used as medicaments in the form of any pharmaceutical formulation. The pharmaceutical formulation can be administered enterally, either orally, e.g. in the form of tablets, coated tablets, dragées, hard and soft gelatine capsules, solutions, emulsions, syrups, or suspensions, or rectally, e.g. in the form of suppositories. They can also be administered parenterally (intramuscularly, intravenously, subcutaneously or intrasternal injection or infusion techniques), e.g. in the form of injection solutions, nasally, e.g. in the form of nasal sprays, or inhalation spray, topically and so forth.

For the manufacture of pharmaceutical preparations, the 4'-substituted nucleoside derivatives, as well as their pharmaceutically useable salts, can be formulated with a therapeutically inert, inorganic or organic excipient for the production of tablets, coated tablets, dragées, hard and soft gelatine capsules, solutions, emulsions or suspensions.

The compounds of formula I can be formulated in admixture with a pharmaceutically acceptable carrier. For example, the compounds of the present invention can be administered orally as pharmacologically acceptable salts. Because the compounds of the present invention are mostly water soluble, they can be administered intravenously in physiological saline solution (e.g., buffered to a pH of about 7.2 to 7.5). Conventional buffers such as phosphates, bicarbonates or citrates can be used for this purpose. Of course, one of ordinary skill in the art may modify the formulations within the teachings of the specification to provide numerous formulations for a particular route of administration without rendering the compositions of the present invention unstable or compromising their therapeutic activity. In particular, the modification of the present compounds to

render them more soluble in water or other vehicle, for example, may be easily accomplished by minor modifications (salt formulation, esterification, etc.) which are well within the ordinary skill in the art. It is also well within the ordinary skill of the art to modify the route of administration and dosage regimen of a particular compound in order to manage the pharmacokinetics of the present compounds for maximum beneficial effect in patients.

For parenteral formulations, the carrier will usually comprise sterile water or aqueous sodium chloride solution, though other ingredients including those which aid dispersion may be included. Of course, where sterile water is to be used and maintained as sterile, the compositions and carriers must also be sterilized. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed.

Suitable excipients for tablets, coated tablets, dragées, and hard gelatin capsules are, for example, lactose, corn starch and derivatives thereof, talc, and stearic acid or its salts.

If desired, the tablets or capsules may be enteric-coated or sustained release by standard techniques.

Suitable excipients for soft gelatine capsules are, for example, vegetable oils, waxes, fats, semi-solid and liquid polyols.

Suitable excipients for injection solutions are, for example, water, saline, alcohols, polyols, glycerine or vegetable oils.

Suitable excipients for suppositories are, for example, natural and hardened oils, waxes, fats, semi-liquid or liquid polyols.

Suitable excipients for solutions and syrups for enteral use are, for example, water, polyols, saccharose, invert sugar and glucose.

The pharmaceutical preparations of the present invention may also be provided as sustained release formulations or other appropriate formulations.

The pharmaceutical preparations can also contain preservatives, solubilizers, stabilizers, wetting agents, emulsifiers, sweeteners, colorants, flavourants, salts for adjustment of the osmotic pressure, buffers, masking agents or antioxidants.

The pharmaceutical preparations may also contain other therapeutically active agents known in the art.

The dosage can vary within wide limits and will, of course, be adjusted to the individual requirements in each particular case. For oral administration, a daily dosage of between about 0.01 and about 100 mg/kg body weight per day should be appropriate in monotherapy and/or in combination therapy. A preferred daily dosage is between about 0.1 and about 500 mg/kg body weight, more preferred 0.1 and about 100 mg/kg body weight and most preferred 1.0 and about 100 mg/kg body weight per day. A typical preparation will contain from about 5% to about 95% active compound (w/w) . The daily dosage can be administered as a single dosage or in divided dosages, typically between 1 and 5 dosages per day.

In certain pharmaceutical dosage forms, the pro-drug form of the compounds, especially including acylated (acetylated or other) derivatives, pyridine esters and various salt forms of the present compounds are preferred. One of ordinary skill in the art will recognise how to readily modify the present compounds to pro-drug forms to facilitate delivery of active compounds to a target site within the host organism or patient. One of ordinary skill in the art will also take advantage of favourable pharmacokinetic parameters of the pro-drug forms, where applicable, in delivering the present compounds to targeted site within the host organism or patient to maximise the intended effect of the compound.

The nucleoside derivatives or the medicaments thereof may be used in monotherapy or combination therapy, i.e. the treatment may be in conjunction with the administration of one or more additional therapeutically active substance(s), for example, an immune system modulator such as an interferon, interleukin, tumor necrosis factor or colony stimulating factor; an antiviral agent or an anti-inflammatory agent. When the treatment is combination therapy, such administration may be concurrent or sequential with respect to that of the 4'-substituted nucleoside derivatives. Concurrent administration, as used herein thus includes administration of the agents at the same time or at different times.

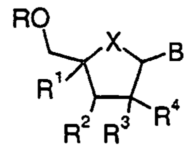
It will be understood that references herein to treatment extend to prophylaxis as well as to the treatment of existing conditions, and that the treatment of animals includes the treatment of humans as well as other mammals. Furthermore, treatment of an Hepatitis C Virus (HCV) infection, as used herein, also includes treatment or prophylaxis of a disease or a condition associated with or mediated by Hepatitis C Virus (HCV) infection, or the clinical symptoms thereof.

In the present specification "comprise" means "includes or consists of" and "comprising" means "including or consisting of".

The features disclosed in the foregoing description, or the following claims, or the accompanying drawings, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for attaining the disclosed result, as appropriate, may, separately, or in any combination of such features, be utilised for realising the invention in diverse forms thereof.

Claims

1. Use of compounds of formula I

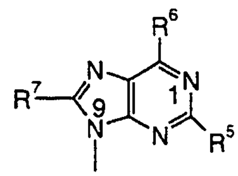


I

wherein

R	is hydrogen or -[P(O)(OH)-O]$_n$H and n is 1, 2 or 3;
R^1	is alkyl, alkenyl, alkynyl, haloalkyl, alkylcarbonyl, alkoxycarbonyl, hydroxyalkyl, alkoxyalkyl, alkoxy, cyano, azido, hydroxyiminomethyl, alkoxyiminomethyl, halogen, alkylcarbonylamino, alkylaminocarbonyl, azidoalkyl, aminomethyl, alkylaminomethyl, dialkylaminomethyl or heterocyclyl;
R^2	is hydrogen, hydroxy, amino, alkyl, hydroxyalkyl, alkoxy, halogen, cyano, or azido;
R^3 and R^4	are hydrogen, hydroxy, alkoxy, halogen or hydroxyalkyl, provided that at least one of R^3 and R^4 is hydrogen; or
R^3 and R^4	together represent $=CH_2$ or =N-OH, or
R^3 and R^4	both represent fluorine;
X	is O, S or CH_2;

B signifies a 9-purinyl residue B1 of formula

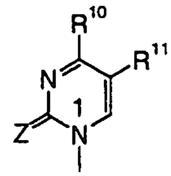


B1

wherein

R^5	is hydrogen, hydroxy, alkyl, alkoxy, alkylthio, NHR^8, halogen or SH;
R^6	is hydroxy, NHR^8, $NHOR^9$, $NHNR^8$, $-NHC(O)OR^{9'}$ or SH;
R^7	is hydrogen, hydroxy, alkyl, alkoxy, alkylthio, NHR^8, halogen, SH or cyano;
R^8	is hydrogen, alkyl, hydroxyalkyl, arylcarbonyl or alkylcarbonyl;
R^9	is hydrogen or alkyl;
$R^{9'}$	is alkyl; and

B signifies a 1-pyrimidyl residue B2 of formula



B2

wherein

Z is O or S;

R^{10} is hydroxy, NHR^8, $NHOR^9$, $NHNR^8$, $-NHC(O)OR^{9'}$ or SH;

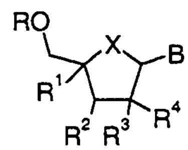
R^{11} is hydrogen, alkyl, hydroxy, hydroxyalkyl, alkoxyalkyl, haloalkyl or halogen;

R^8 R^9 and $R^{9'}$ are as defined above;

and of pharmaceutically acceptable salts thereof;

for the treatment of diseases mediated by the Hepatitis C Virus (HCV) and for the preparation of a medicament for such treatment.

2. The use according to claim 1 of compounds of formula I



I

wherein

R is hydrogen;

R^1 is alkyl, alkenyl, alkynyl, haloalkyl, alkylcarbonyl, alkoxy, hydroxymethyl, cyano, azido, alkoxyiminomethyl, alkylcarbonylamino, alkylaminomethyl or dialkylaminomethyl;

R^2 is hydrogen, hydroxy, alkoxy, or halogen;

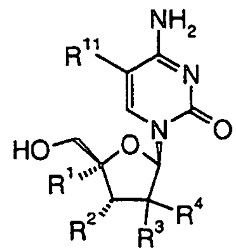
R^3 and R^4 are hydrogen, hydroxy, alkoxy, halogen or hydroxyalkyl, provided that at least one of R^3 and R^4 is hydrogen; or

R^3 and R^4 represent fluorine;

X is O or CH_2; and

B signifies a 9-purinyl residue B1 or a 1-pyrimidyl residue B2 as defined in claim 1.

3. The use according to claim 1 or claim 2 of the compounds of formula



I-a

wherein

R^1	is alkyl, alkenyl, alkynyl, haloalkyl, alkylcarbonyl, alkoxy, hydroxymethyl, cyano, azido, alkoxyiminomethyl, alkylcarbonylamino, alkylaminomethyl or dialkyl-aminomethyl;
R^2	R^2 is hydrogen, hydroxy, alkoxy, or halogen;
R^3 and R^4	are hydrogen, hydroxy, alkoxy, halogen or hydroxyalkyl, provided that at least one of R^3 and R^4 is hydrogen; or
R^3 and R^4	represent fluorine.

and pharmaceutically acceptable salts thereof.

4. The use of a compounds according to claim 3, wherein the compounds are
4'-C-ethynylcytidine hydrochloride (1:1)
4'-C-ethoxycytidine
4'-C-acetylcytidine

5. The use of a compound according to claim 3, wherein the compound is
4'-C-azidocytidine

6. A compound as defined in any one of claims 1 to 5 or a pharmaceutically acceptable salt thereof for the treatment of diseases mediated by the hepatitis C virus (HCV).

7. A compound as defined in any one of claims 1 to 5 or a pharmaceutically acceptable salt thereof for the preparation of medicaments for the treatment of diseases mediated by the hepatitis C virus (HCV).

8. A pharmaceutical composition on the basis of a pharmaceutically effective amount of a compound of formula I or I-a or a pharmaceutically acceptable salt thereof, as defined in any one of claims 1 to 5 for the treatment of diseases

mediated by the hepatitis C virus (HCV) or for the preparation of a medicament for such treatment.

9. The use of a pharmaceutical composition on the basis of a pharmaceutically effective amount of a compound of formula I or I-a or a pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 5 for the treatment of diseases mediated by the hepatitis C virus (HCV).

10. The invention as hereinbefore described.